lation. Earlier work by Charbon [see (*1*, *2*)] showed that intravenous injections of parathyroid extract increased blood flow in the coeliac and renal circulations of the dog. More recently, Charbon and Hulstaert (*3*) demonstrated that the Beckman synthetic bPTH(1–34) peptide, possessing a lower activity (2846 IU/mg), could cause a four- to fivefold increase in hepatic artery blood flow. Thus the bPTH(1–34) vasodilatory response is not limited to the coronary circulation. The hypotensive responses elicited by bPTH(1–34) in the present studies implied a generalized vasodilatory effect. Also, bPTH(1–34) has been shown to increase blood flow rate in dog kidneys and rat hind limbs and to relax helical strips of rabbit aorta (*4*). Of interest has been the inability to block the blood pressure-lowering effect of bPTH(1–34) in dogs and rats with α- or β-adrenergic, cholinergic, or histaminergic blocking drugs (*4*). This suggests to us that the coronary vasodilatory response to synthetic bPTH(1–34) is probably a direct one, involving specialized receptors on the vascular smooth musculature.

The vasodepressor response to bPTH(1–34) has been demonstrated in a variety of species and preparations by Pang *et al.* (*4*), including South American lungfish, bullfrog, water snake, domestic chicken, and rat.

Our findings with a pure synthetic form of a naturally occurring polypeptide hormone may be useful in understanding the regulation of coronary smooth muscle tone. The fact that a known primary function of parathyroid hormone is the regulation of blood calcium level, coupled with the well-known importance of calcium in the control of vascular smooth muscle contractility, lends further significance to the present observations.

M. F. CRASS, III
PETER K. T. PANG
Departments of Physiology and *Pharmacology, School of Medicine, Texas Tech University Health Sciences Center, Lubbock 79430*

References and Notes

1. G. A. Charbon, *Eur. J. Pharmacol.* **3**, 275 (1968).
2. _____ and E. E. M. Pieper, *Endocrinology* **91**, 828 (1972).
3. G. A. Charbon and P. F. Hulstaert, *ibid.* **95**, 621 (1974).
4. P. K. T. Pang, T. E. Tenner, Jr., J. A. Yee, M. Yang, H. F. Janssen, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 675 (1980).
5. We thank D. J. Paulson and E. Roy for excellent technical assistance.

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Identification of Mosquitoes of *Anopheles gambiae* Species Complex A and B by Analysis of Cuticular Components

Abstract. *Two important vectors of malaria in Africa,* Anopheles gambiae *and* Anopheles arabiensis *(Diptera: Culicidae), often occur sympatrically and cannot be distinguished morphologically. A chemical method was developed to identify individual laboratory-reared adult males or females of either species by extraction and analysis of cuticular components with gas chromatography. Statistically significant differences were seen between species when selected pairs of peaks were compared.*

The *Anopheles gambiae* Giles complex of Africa includes two species that breed in salt water, namely *An. melas* Theobald in West Africa and *An. merus* Donitz in East Africa. Three more species, which breed in freshwater, were previously designated as species A, B, and C and are now designated *An. gambiae* Giles, *An. arabiensis* Patton, and *An. quadriannulatus* Theobald (*1*, *2*), respectively. A fourth member, species D (Bwamba cytotype), is found only in waters with high mineral contents in the Semliki forest of Uganda (*3*). The two most important species, *An. gambiae* (A) and *An. arabiensis* (B), are highly anthropophilic, are the principal vectors of malaria in the Afrotropical region, and occur sympatrically in many areas. They are true sibling species in which interbreeding gives sterile males, and they can only be separated with confidence by the banding of the polytene chromosomes of either the fourth-instar larval salivary glands (*4*) or the adult ovarian nurse cells of half-gravid females (*5*). Recently, however, a method has been described for identifying larvae and pupae of these two species by fluorochrome bisbenzimid staining of the mitotic karyotypes (*6*). In another procedure that has been tried, diagnostic allozymes are used for separating certain species of the *gambiae* complex (*7*, *8*).

The usefulness of these methods for work in the field requires further evaluation because each has severe technical limitations. For example, only females can be identified by chromosomal studies, and they must be half-gravid. In addition, cytotaxonomic identifications are difficult on preserved material with the result that many individuals cannot be identified (*9*). For allozyme studies it is necessary to keep field-collected samples continuously frozen in Dry Ice from time of collection until laboratory analysis.

We therefore investigated alternative methods of identifying adults of either sex of *An. gambiae* and *An. arabiensis*. Extraction and analysis of chemically stable and easily obtained cuticular hydrocarbons was carried out by gas chromatography (GC) to discover whether there were specific ingredients or unique relationships that could serve as markers. We report here some minor but statistically significant differences in the cuticular components of laboratory-reared adults of these two sibling species.

Sample of 2- to 3-week-old unmated mosquitoes were obtained from laboratory colonies of *An. gambiae* and *An. arabiensis* maintained at the Liverpool School of Tropical Medicine in England.

Crude lipids were extracted from cuticular samples of individuals or pooled samples of each sex and species by soaking each sample twice in a minimum of *n*-heptane. The extract was transferred to a clean glass vial, and the solvent was removed for shipment by evaporation with a stream of dry air. Crude extracts were redissolved in a minimum amount of hexane (10 μl) for immediate GC analysis (Fig. 1) or added to a column prepared from a disposable Pasteur pipette (5 mm in diameter), dry-packed with 60 to 200 mesh silica gel (2 cm) over 20 percent silver nitrate–impregnated silica gel (1 cm). Saturated paraffins were eluted with *n*-hexane, olefins with 1 percent ether in hexane, and polyolefins with 15 percent ether in hexane. The volume of each separated sample was adjusted to 10 μl for GC analysis (*10*).

The major constituents of the crude lipid extract were *n*-paraffins of 21 to 33 carbons and branched paraffins of 29 to 44 carbons, consistent with a previous analysis of pooled samples of 500 mosquitoes (*11*). Patterns in the chromatograms of components with 21 to 44 carbons appeared to be similar in all samples. Also in this range were smaller peaks, but none that appeared to be unique to either species or either sex. Saturated or unsaturated hydrocarbons that might be species markers were not found in sufficient quantities to be useful.

Quantitative data on the distribution of larger peaks suggests that there are at least three relationships that can be used for identification markers of females of

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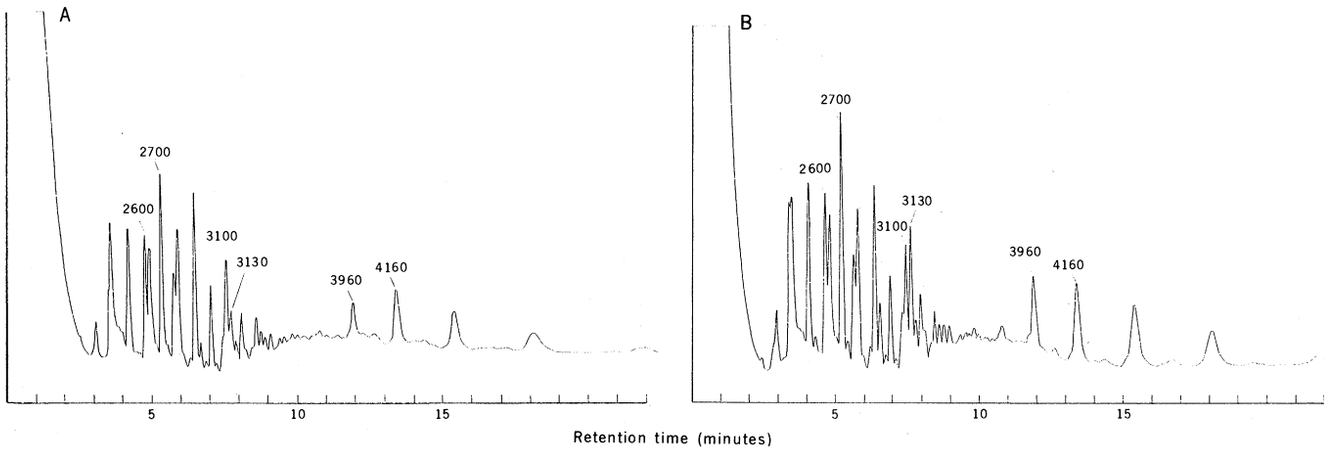

Fig. 1. Gas chromatograms of crude extracts from individual female *Anopheles gambiae* complex mosquitoes. (A) *Anopheles gambiae*. (B) *Anopheles arabiensis*.

these two species. One marker is the relative abundance (R) of 26 and 27 carbon paraffins, that is, compared to n-heptacosane (KI 2700), the R of n-hexacosane (KI 2600) was 0.69 ± 0.07 in crude extracts of single female *An. gambiae* but only 0.55 ± 0.10 in female *An. arabiensis* (Table 1). The ratios were significantly different by analysis of variance ($P < .05$) (*12*).

A second marker is R of peaks that elute with 13-methylhentriacontane (KI 3130) compared to n-hentriacontane (KI 3100). The values for R for crude extracts of single females, 0.30 ± 0.22 for *An. gambiae* and 0.79 ± 0.29 for *An. arabiensis*, were highly significantly different ($P < .005$).

A third marker is the relative abundance of dimethylnonatriacontane (KI 3960) compared to dimethylhentetracontane (KI 4160) (*13*). The values for R in crude extracts of single females, 0.54 ± 0.08 for *An. gambiae* and 0.81 ± 0.07 for *An. arabiensis*, were highly significantly different ($P < .005$). The elements in this set of values were well separated, and no overlapping values were seen.

Silica gel and argentation-liquid chromatography was conducted in attempts to improve resolution of peaks. Removal of nonparaffins by chromatography did not improve the analysis, as statistical significance declined between R values for the first marker (2600/2700) in paraffins from individual and pooled samples of females (Table 1). However, the differences in the second and third markers remained significant ($P < .05$, $P < .01$) in paraffins from individual females and in paraffins from pooled females ($P < .05$, $P < .005$), respectively.

Crude cuticular extracts from single males were evaluated by the same procedures, and 2600/2700, 3130/3100, and 3960/4160 R values were determined to be significantly different at .05, .005, and .05 levels, respectively (Table 1). With removal of nonparaffins, only the 3960/4160 values remained highly significantly different ($P < .005$) in single males, while significant differences were retained in pooled paraffin samples ($P < .005$) for both the 2600/2700 and 3960/4160 values.

A correct identification could usually be made by observation of the chromatograms of crude extracts when unidentified nonparaffins contributed to peak height. Use of an automated data system is not obligatory because simple measurement of GC peak heights from the strip-chart recorder gave the same relative information used for 3130/3100 values in some samples.

It is more important to differentiate females than males because the females are vectors of disease, and females are more often caught than males. However, this simple technique was also successful when applied to males, even in the worst case when two of the three values were not significantly different. For example, when Fisher's method of combining tests (*14*) was applied to all three values of R for paraffins from individual males, the significance was reduced slightly ($P < .05$).

This technique is potentially useful for field-workers because cuticular hydrocarbons are chemically very stable, and it may be possible to utilize adults from light-trap collections, dead adults that are rather old, or even pinned specimens. To be most useful, the values obtained should be consistent over the range of the species. Although some variation between conspecifics may be expected, values found with individual mosquitoes may be consistent enough to observe with confidence values obtained for pooled samples (*11, 15*).

Table 1. Identification of *An. gambiae* and *An. arabiensis* by gas chromatography. Means and standard deviations are presented for relative abundances (R) for females and males. Peaks were measured or quantitated electronically, and three pairs of peaks were selected and compared for each specimen or pooled sample.

R 2600/2700		R 3130/3100		R 3960/4160	
An. gambiae	*An. arabiensis*	*An. gambiae*	*An. arabiensis*	*An. gambiae*	*An. arabiensis*
Females: peaks in extracts of individuals (N = 7)					
0.69 ± 0.07	0.55 ± 0.10*	0.30 ± 0.22	0.79 ± 0.29†	0.54 ± 0.08‡	0.81 ± 0.07†
Paraffins from individuals (N = 5)					
0.42 ± 0.14	0.32 ± 0.11	1.00 ± 0.29§	1.58 ± 0.47*	0.41 ± 0.08	0.63 ± 0.10‖
Paraffins from pooled samples (N = 5)					
0.31 ± 0.06	0.18 ± 0.21	1.82 ± 0.59§	3.13 ± 0.80‡*	0.42 ± 0.01	0.73 ± 0.06†
Males: peaks in extracts of individuals (N = 6)					
0.74 ± 0.09	0.56 ± 0.13*	0.30 ± 0.09	0.92 ± 0.19†	0.51 ± 0.23‡	0.74 ± 0.14*
Paraffins from individuals (N = 5)					
0.37 ± 0.11	0.38 ± 0.15	1.24 ± 0.28§	1.11 ± 0.31§	0.41 ± 0.05	0.64 ± 0.10†
Paraffins from pooled sample (N = 5)					
0.49 ± 0.10	0.19 ± 0.11†	1.36 ± 1.41§	2.12 ± 0.96§	0.41 ± 0.09	0.80 ± 0.08†

*R values significantly different ($P < .05$). †R values highly significantly different ($P < .005$). ‡N = 9. §Determined by peak height measurement. ‖R values highly significantly different. ($P < .01$).

These results merit detailed appraisal of the value of the cuticular components of wild adults for the separation of these and other sibling species of the *An. gambiae* complex, and perhaps other complexes, for example, species of the *Culex pipiens* or *Aedes aegypti*. Examination of materials from these and other mosquito species with the same techniques showed dissimilar distribution of components, suggesting that some cuticular components may possess biological activity related to species or sex recognition, or both. There is ample precedent for this in higher Diptera, because relatively nonvolatile cuticular hydrocarbons are responsible for male sexual stimulation and sex recognition in the tsetse fly (*16*) among others.

D. A. CARLSON
Insects Affecting Man and Animals Research Laboratory, Agricultural Research, Science and Education Administration, U.S. Department of Agriculture, Gainesville, Florida 32604
M. W. SERVICE
Department of Medical Entomology, Liverpool School of Tropical Medicine, Liverpool L3 5QA England

References and Notes

1. G. B. White, *Mosq. Syst.* **7**, 303 (1975).
2. M. W. Service, *ibid.* **8**, 217 (1976).
3. G. B. White, *Trans. R. Soc. Trop. Med. Hyg.* **68**, 278 (1974).
4. M. Colluzi and A. Sabatini, *Parassitologia* **9**, 73 (1967).
5. M. Colluzi, *ibid.* **10**, 179 (1968).
6. M Gatti, G. Santini, S. Pimpinelli, M. Colluzi, *Heredity* **38**, 105 (1977).
7. R. J. Mahon, L. A. Green, R . H. Hunt, *Bull. Entomol. Res.* **66**, 25 (1976).
8. S. J. Miles, *ibid.* **68**, 85 (1978).
9. M. W. Service, G. P. Joshi, G. D. Pradhan, *Ann. Trop. Med. Parasitol.* **72**, 377 (1978).
10. The gas chromatograph (Varian model 1440) was equipped with a flame ionization detector, a Hewlett-Packard model 3380A integrator, and a 1.8 m by 2 mm (inner diameter) glass column packed with nonpolar 3 percent OV-1 on 100 to 120 mesh Gas Chrom Q. Samples were analyzed with the column oven temperature programmed to increase from 200° to 335°C at 12°C per minute. Other parameters were: injector port, 310°C; detector, 385°C. The carrier gas was helium, 20 ml/min. Paraffin standards (Analabs) were used to ensure that the correct GC peaks were being quantitated. Kovats indices (KI values) were assigned to compounds eluting from a nonpolar GC column held at one temperature based on their retention times by using a log scale, relative to straight-chain (normal) saturated paraffins [E. Kovats, *Adv. Chromatogr.* **1**, 229 (1965)]. Thus a compound that coelutes with *n*-hentriacontane (nC_{31}) was assigned KI 3100. One methyl branch added to the center carbon of a chain will typically add 30 units, that is, 13-methylhentriacontane had a KI of 3130. The first fraction (50 ml of hexane eluted from a column of silver nitrate–silica gel) contained only saturated hydrocarbons for which KI values were dependent only on the number and location of branches on the chain and possibly the length of the branches.
11. D. A. Carlson and M. W. Service, *Ann. Trop. Med. Parasitol.* **73**, 589 (1979).
12. R. G. Steel and J. H. Torrie, *Principles and Procedures of Statistics* (McGraw-Hill, New York, 1960).
13. Combined gas chromatography–mass spectrometry studies were conducted with a Varian 112 mass spectrometer interfaced to a Varian 3700 gas chromatograph fitted with a 3.6 m by 2 mm column of OV-101 temperature programmed to increase at 3°C per minute from 160° to 320°C. Peaks at KI 2600, 2700, and 3100 were *n*-paraffins in *An. arabienis* females. The peak at KI 3130 in crude extracts was a mixture simplified by chromatography to leave two paraffins, 13- and 15-methylhentriacontane, with fragments at mass to charge (*m/e*) ratios of 196, 224, 252, and 280. The peak at KI 3960 contained 13,21-dimethylonatriacontane, which yielded fragments at *m/e* 196, 280, and 407, and less of the 15,23 isomer, which yielded fragments at *m/e* 224, 252, and 379. The peak at KI 4160 contained 13,21-dimethylhentetracontane, which yielded fragments at *m/e* 196, 308, and 435, and less of the 15,23-isomer, which yielded fragments at *m/e* 224, 280, and 407.
14. R. A. Fisher, *Statistical Methods for Research Workers* (Oliver & Boyd, ed. 11, London, 1950).
15. Several precautions were taken: polyethylene vial caps were scrupulously avoided, and GC column bleed was minimized by use of OV-1 liquid phase in a carefully maintained glass column that was periodically treated with silylating agent to maintain about 3600 theroretical plates, *n*-pentatriacontane (KI 3500) being used for this calculation. An aluminum lining was used on the inner face of the GC septa to keep peaks from septum purge much smaller than the small peaks obtained from single mosquitoes.
16. D. A. Carlson, P. A. Langley, P. Huyton, *Science* **201**, 750 (1978).
17. We thank N. Chen-Langenmayr for technical assistance, J. Seawright, M. Huettel, D. Joslyn, and L. Mukwaya for comments, C. Fatland for mass spectra, and D. Nelson for discussion.

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Renin-Specific Antibody for Study of Cardiovascular Homeostasis

Abstract. *Antiserum specific for purified canine renal renin was used to inhibit this enzyme in trained, conscious dogs. The antiserum did not affect blood pressure in sodium-replete dogs but decreased plasma renin activity and blood pressure in sodium-depleted animals. The antiserum also reduced blood pressure to control levels concomitant with suppression of plasma renin activity in uninephrectomized dogs with acute renovascular hypertension. These observations establish the role of the renin-angiotensin system in the maintenance of blood pressure in the sodium-depleted state as well as in the initiation of renovascular hypertension.*

The role of the renin-angiotensin system in blood pressure homeostasis and in the pathogenesis of renovascular hypertension is not completely understood. Data derived from pharmacological blockade of this system by means of angiotensin I converting enzyme inhibitors or angiotensin II antagonists are inconclusive because of the bradykinin potentiating effect of the former (*1, 2*) and the partial agonistic property of the latter (*3*). Immunologic blockade experiments in rats and rabbits given antibodies to purified converting enzyme (*4, 5*) or angiotensin II (*6–8*) have yielded some insights. Since renin is the enzyme responsible for the initial step in the generation of angiotensin II, specific inhibition at this site would be the preferable method of blockade. Results of previous experiments (*9–11*) have been equivocal because purified renin for antibody production has not been available. We undertook the purification of dog renal renin and the production of antibodies for specific renin blockade in the conscious dog.

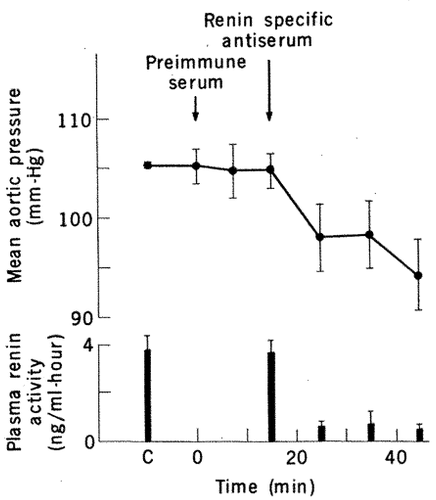

Fig. 1. In salt-depleted dogs, preimmune serum had no effect whereas renin-specific antiserum lowered plasma renin activity, causing blood pressure to fall.

Canine kidneys were subjected to an eight-step purification procedure (*12*). Renal cortex was minced, lyophilized, and pulverized, and the renin was extracted with acetone and water. The extract was batch concentrated on diethylaminoethyl cellulose, acidified to *p*H 3.0, and subjected to sodium chloride and ammonium sulfate precipitation. Renin was then separated from other proteases by using a linear concentration gradient on carboxymethyl cellulose chromatography. Pepstatin affinity chromatography (*13*) then resulted in a 300-fold purification. Finally, gel filtration on Sephadex G-100 yielded a single symmetrical peak of renin activity of 4200 Goldblatt units per milligram (*14*) of protein and a recovery of 17 percent with an overall purification of 600,000-fold. Polyacrylamide gel electrophoresis demonstrated a single homogeneous band. When slices from a simultaneously run unstained gel were assayed, a discrete peak of renin activity, as measured by the method of Haber *et al.* (*15*), was recovered in a position corresponding to the stained band. So-

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