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.

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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.
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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.
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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.
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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.

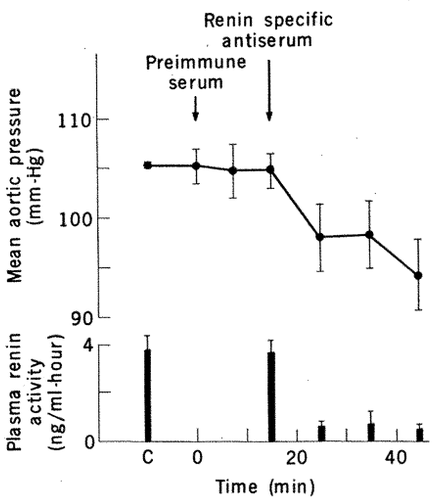

Fig. 1. In salt-depleted dogs, preimmune serum had no effect whereas renin-specific antiserum lowered plasma renin activity, causing blood pressure to fall.

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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dium dodecyl sulfate (SDS)-gel electrophoresis also showed a single protein band.

Antibodies to dog renin were obtained by injecting goats intramuscularly and intradermally with 250 μg of the purified enzyme. Monthly booster injections of 100 μg of renin were administered at multiple intradermal sites. High-titer antiserum was obtained. At a dilution of 1:20,000, 100 μl of antiserum resulted in 50 percent inhibition of the enzymatic activity of 0.002 Goldblatt unit of standardized dog renin. When subjected to immunodiffusion and immunoelectrophoresis, the antiserum formed a single precipitin band with crude kidney extract; this band was identical to that obtained with pure renin. These specific antibodies to renin were then used for immunologic blockade experiments in the conscious dog. One unit of renin antiserum was the amount of serum required to inhibit completely in vitro the renin activity of 1 Goldblatt unit of standardized dog renin.

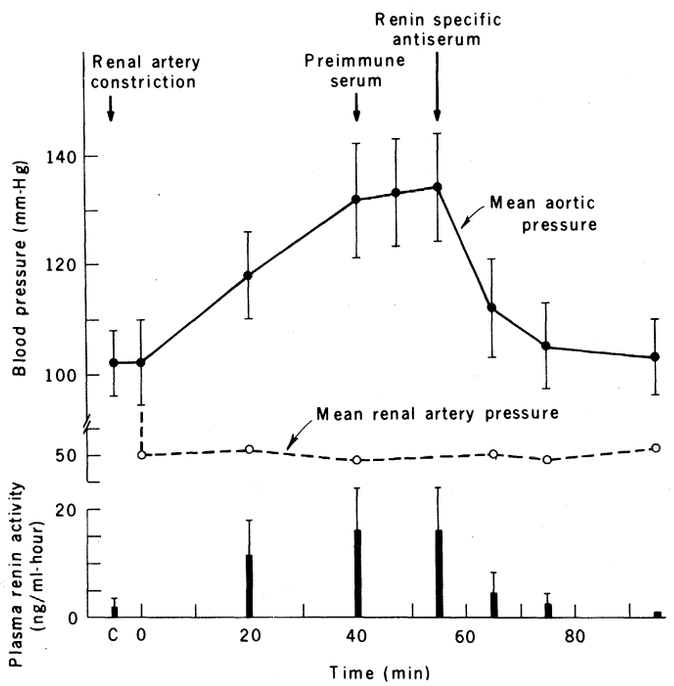
Dogs were trained to lie quietly on a padded table. They were then anesthetized and subjected to unilateral nephrectomy under sterile conditions. Polyvinyl catheters were implanted into the aorta, renal artery, and inferior vena cava and the ends exteriorized. An externally inflatable Silastic constricting cuff was placed around the renal artery proximal to the renal artery catheter. In some animals, an electromagnetic flow probe was secured around the origin of the renal artery. Experiments on these dogs were started 2 weeks after surgery. Systemic and renal arterial pressures were monitored with P23 Statham pressure transducers and recorded with an electromagnetic flow meter. Blood samples were collected for determination of plasma renin activity (PRA) by the radioimmunoassay of Haber *et al.* (*15*) and expressed as nanograms of angiotensin I generated per milliliter per hour. Purified renin and antiserums were administered as single bolus injections through the catheter implanted in the inferior vena cava. Renal vascular resistance was calculated by dividing mean renal arterial pressure by renal blood flow.

One Goldblatt unit of purified dog renin given intravenously resulted in a 30 mm-Hg rise in mean aortic pressure (MAP) and an approximately 50 percent increase in renal vascular resistance. These effects were completely blocked by renin specific antiserum. The antibodies had no effect on the pressor response to systemic administration of 2 μg of angiotensin I or angiotensin II.

The basal PRA of three dogs maintained on a diet containing 80 milliequivalents of sodium and 60 meq of potassium was 0.4 ng/ml-hour. Six units of renin antiserum (7.5 ml) had no significant effect on MAP in the salt-replete state. Similarly, the nonapeptide angiotensin I converting enzyme inhibitor (<Glu-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro) resulted in only a transient decrease in blood pressure of 5 mm-Hg in these animals.

Four dogs were placed on a diet containing 10 meq of sodium and 60 meq of potassium and given 80 mg of Furosemide orally for 5 days. The urinary sodium excretion at steady state was 1 meq/day and the PRA increased to 3.8 ± 0.4 ng/ml-hour. An intravenous bolus of converting enzyme inhibitor (5 mg) decreased MAP by an average of 12 mm-Hg in these dogs. Administration of 7.5 ml of preimmune serum (obtained from nonimmunized goats) had no effect on PRA or MAP. Six units of antiserum (7.5 ml), an amount calculated to be in considerable excess of that needed to neutralize circulating renin in all experiments performed, decreased PRA from 3.8 ± 0.4 to < 0.4 ng/ml-hour, and was paralleled by a prompt decrease in MAP from 105 ± 2 to 94 ± 4 mm-Hg within 30 minutes (Fig. 1). The effectiveness of renin blockade was evidenced by the lack of a pressor response to exogenous renin.

Renovascular hypertension was induced in five days by inflation of the Silastic cuff which reduced renal artery pressure to 50 mm-Hg (Fig. 2). The PRA increased from 3.4 ± 1.8 to 18.3 ± 6.3



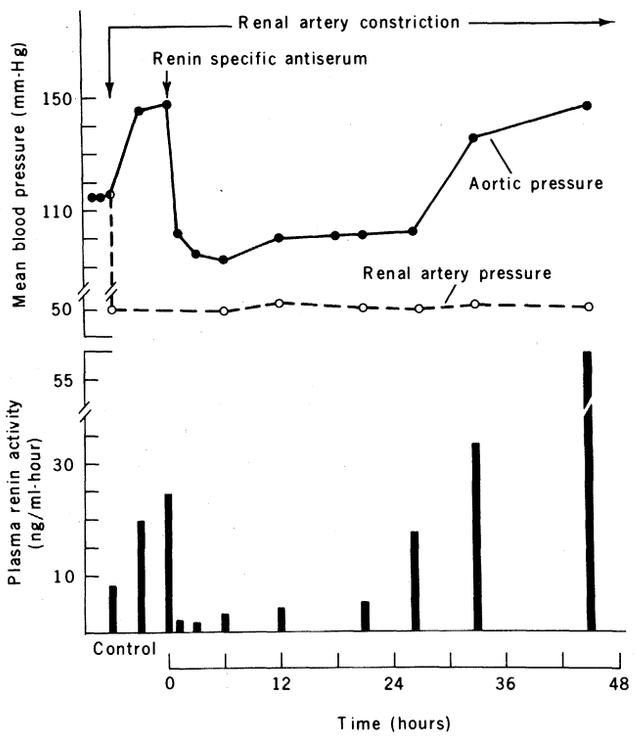


Fig. 2 (left). After renal artery constriction, preimmune serum had no significant effects whereas specific antiserum resulted in a prompt return of aortic pressure and plasma renin activity to control levels. Fig. 3 (right). A representative experiment illustrating the duration of action of the antiserum. After renovascular hypertension was produced, administration of renin-specific antiserum resulted in a sustained suppression of systemic blood pressure and plasma renin activity below control levels for 24 hours.

ng/ml-hour within 40 minutes. Simultaneously, MAP increased from a control level of 102 ± 6 to a plateau of 134 ± 7 mm-Hg and renal vascular resistance decreased by 11 percent. Administration of 7.5 ml of preimmune serum had no significant effect on these measures. Six units of renin-specific antiserum given 1 hour after constriction caused PRA to fall to control levels in 10 minutes and to 1.3 ± 0.7 ng/ml-hour in 40 minutes. The decrease in PRA was accompanied by a similar reduction in MAP to a nadir of 102 ± 6 mm-Hg and a 40 percent further decrease in renal vascular resistance.

The duration of action of the antibody was at least 21 hours as evidenced by continued suppression of PRA and MAP at or below control levels despite maintenance of renal perfusion pressure at 50 mm-Hg. Beyond this time PRA and MAP slowly approached their postconstriction, preantiserum levels (Fig. 3). This observation regarding duration of action was further supported by the inability of exogenous renin to increase systemic blood pressure for the first 24 hours. Injection of 1 Goldblatt unit of renin thereafter resulted in a pressor response comparable to that obtained before antiserum administration.

Renin-specific antibody completely blocked the pressor action of endogenous and exogenous dog renin, but had no effect on the pressor actions of angiotensin I or angiotensin II. The antibody caused no significant change in mean blood pressure in the sodium-replete state. In the sodium-depleted state, however, with consequent increased renin activity, the binding of the antibody to the enzyme reduced PRA below control levels. This was accompanied by a significant fall in blood pressure. After renal artery constriction, the rise in systemic pressure was associated with an increase in PRA. Renin antibody lowered PRA and restored pressure to normal. The onset of action of the antiserum was prompt (minutes) and the duration of action prolonged (approximately 24 hours). Since the antibody acts by inhibiting renin's enzymatic activity and does not appear to have other pharmacologic actions, these results provide definitive evidence for the role of the renin-angiotensin system in blood pressure homeostasis during sodium-depletion and in the initiation of renovascular hypertension.

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Lateralization of Reward in Rats: Differences in Reinforcing Thresholds

Abstract. *Fourteen rats with bilaterally implanted lateral hypothalamic electrodes were allowed to self-stimulate each side of the brain during daily test sessions. Rotation (circling behavior) during self-stimulation sessions was also recorded. All rats rotated in a preferential direction regardless of the side of the brain stimulated, and, in each case, the direction was the same as that subsequently determined in response to* d*-amphetamine. All rats had asymmetries in self-stimulation thresholds related to the direction of rotation. Thresholds were lower on the side contralateral to the direction of rotation, and entire rate-intensity functions were displaced to the left on that side. The results, discussed in terms of lateralization of affect, suggest a model in which quantitative differences in neuronal firing can be translated into apparent qualitative specialization, with the two sides of the brain appearing to be specialized for high and low mood, respectively.*

Cerebral functional asymmetry, once considered a unique characteristic of the human brain (*1*), has now been demonstrated in the brains of various animal species, including other primates (*2*), cats (*3*), rodents (*4*), and songbirds (*5*). Research conducted in our laboratory has established that normal rats have an asymmetry in nigrostriatal function; asymmetries in striatal dopamine content (*6*), striatal dopamine metabolism, and dopamine-stimulated adenylate cyclase activity (*7*) have been related to spontaneous side preferences (*6*) and to nocturnal (*8*) and drug-induced (*9*) circling behavior. Recently, using labeled deoxy-D-glucose to assess glucose utilization in rats (*10*), we reported evidence of asymmetry in several brain regions (*11*)—the results suggested stronger similarities in lateralization of human and rat brains than had previously been envisioned. We have now investigated the possibility of lateralized affect in rats. Neurological findings indicate that the two sides of the human brain are specialized in this regard, with one hemisphere characterized as more joyful and the other as more depressive (*12*). Reasoning that differences in affect could result from differences in the activity of mechanisms mediating reinforcement, we speculated that the two sides of the rat brain might be differentially sensitive to reinforcing brain stimulation.

The subjects were 14 naïve female Sprague-Dawley rats approximately 3 months old and weighing 250 to 280 g. All rats were initially administered *d*-amphetamine sulfate (1.0 mg per kilogram of body weight, injected intraperitoneally) and placed individually in an automated apparatus (*13*) in which circling behavior (or rotation) was measured for 1 hour. Surgery was performed at least a week later. Bipolar stainless steel electrodes were stereotaxically (*14*) implanted in both lateral hypothalami (*15*) of each rat. Electrode placements were verified histologically after the experiment was completed (*16*).

Testing for self-stimulation was first begun 3 to 4 days after surgery. Rats were placed in a Plexiglas cylinder (30 cm in diameter) containing a single lever and enclosed in a sound-attenuated cubicle. Lever-press responses were continuously rewarded with electrical stimulation to the lateral hypothalamus (one side at a time). The stimulation was a 60-

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