acid) τ 6.5 (singlet). Ultraviolet absorption in 0.1*N* HCl showed peaks (λ_{max}) at 231 nm ($\epsilon = 10{,}700$); and at 284 nm ($\epsilon = 9{,}650$). In 0.1*N* NaOH, λ_{max} was 269 nm ($\epsilon = 10{,}150$) and at 309 nm ($\epsilon = 12{,}800$).

12. The data for 1-methylhydantoin-5-oxime are as follows. Calculated (percent) for $C_4H_5N_3O_3$: C, 33.57; H, 3.50; N, 29.37. Values (percent) found were C, 33.30; H, 3.54; N, 29.36. The mass spectrum [*m/e* (relative intensity)] indicated 143 [parent mass ion (23)], 127 (11); 126 (50); 83 (15); 72 6(4); 70 (44); 57 (11); 56 (47); 55 (32); 54 (23); 53 (20); 43 (27); 42 (100); 41 (12); 30 (18). The infrared data (cm^{-1}) showed 3260 (s, broad); 1780 (m); 1730 (s); 1655 (s); 1440 (s); 1240 (w); 1130 (m); 1070 (m); 1000 (s) NMR (in D_2O/NaOD) showed τ 6.55 (singlet); (in trifluoroacetic acid) τ 6.9 (singlet). Ultraviolet absorption in 0.1*N* HCl showed λ_{max} at 225 nm ($\epsilon = 6300$); 283 nm ($\epsilon = 5750$). In 0.1*N* NaOH, λ_{max} was 259 nm ($\epsilon = 9000$); 311 nm ($\epsilon = 8850$).

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Isolation and Characterization of Larvicidal Principle of Garlic

Abstract. *The larvicidal principles of garlic,* Allium sativum *L., have been isolated and identified as diallyl disulfide and diallyl trisulfide. Both natural and synthetic samples of these larvicides are fatal at 5 parts per million to* Culex pipiens quinquefasciatus *Say.*

Active search for effective plant agents that will destroy mosquitoes has been prompted by the controversy concerning the general harmfulness of DDT and by the development by insect pests of resistance to various other chemical insecticides. At one time DDT was considered a panacea for eliminating the mosquito problem. Although medicinal and antibacterial properties of garlic (*Allium sativum* L., N.O. Liliaceae) have been extensively studied (*1*), only recently has the larvicidal property of its oil, for at least four species of mosquitoes in *Culex* and *Aedes* genera (*2*), been demonstrated. Greenstock (*3*) has shown that garlic oil could destroy aphids, cabbage-white butterfly caterpillars, and Colorado beetle larvae. We now report the isolation, characterization, and testing of the active principle in garlic responsible for the mosquito control. We used *Culex pipens quinquefasciatus* Say (the same as *C.p. fatigans* Wiedemann) as test organism.

The medicinal properties of garlic and related plants have prompted study of their chemical composition (*4*). Several *S*-substituted cysteines and cysteine sulfoxides, partly in the free form and partly as γ-glutamyl peptides have been isolated from various species of *Allium*. However, it has been proved that the physiologically active compounds are formed through the enzymatic reactions and spontaneous decomposition of parent compounds. The alkyl sulfides, cysteine sulfoxides, and thiols that have been reported to be present are produced by the degradations of the precursors (*1*).

In our work the crude garlic oil was obtained by steam distillation of homogenized garlic cloves. The oil was purified on a silica gel column and was eluted with solvents of increasing polarity. The fractions obtained from the column were tested for larvicidal activity as described (*2*). The fractions eluting with light petroleum had pronounced larvicidal activity. The active fraction contained sulfur. The infrared spectrum (1640, 990, and 910 cm^{-1}), nuclear magnetic resonance (NMR) spectrum (3.5 δ, 2H; 5.25 δ, 2H; 5.98 δ, 1H), and mass (*m/e*, 41) spectrum show the presence of allyl ($CH_2=CH-CH_2-$) group. Color reactions and absence of lowfield signal in the NMR spectrum indicated the absence a thiol group. Since there are no sulfur-oxygen absorptions (*5*) in the infrared spectrum, sulfur should be present as sulfide linkage only. Gas-liquid chromatography (GLC) (*6*) indicated the presence of several components. Two major components could be separated by preparative GLC and were subjected to mass spectroscopy. The more volatile component was identified as diallyl disulfide (*m/e*, 146) while the other fraction corresponded to diallyl trisulfide (*m/e*, 178). A trace amount of dialyl tetrasulfide (*m/e*, 210) was also indicated. The above conclusions were confirmed by comparison with synthetic preparations. Diallyl disulfide was prepared as was described by Carson and Wong (*7*). A mixture of diallyl disulfide and diallyl trisulfide was obtained by the interaction of sodium polysulfides and allyl bromide. Diallyl trisulfide could be separated from the mixture by preparative GLC (*6*). The presence of diallyl disulfide and diallyl trisulfide in the natural sample was confirmed by the infrared and mass spectra and GLC comparisons with the synthetic samples.

The larvicidal action of the natural samples has been compared to several synthetic samples as shown in Table 1. The relative effectiveness of diallyl disulfide and diallyl trisulfide alone or in mixture even at a concentration of 5 ppm, as against the ineffectiveness of the related compounds diallyl sulfide and dipropyl disulfide and dipropyl trisulfide at 200 ppm is noteworthy. We have also observed that antagonistic properties of diallyl di- and trisulfides against several pests of economic and medical importance such as potato tuber moth, red cotton bug, red palm weevil, houseflies, and mosquitoes. The nontoxic nature of garlic to higher animals has been established on the basis that it has been used for edible pur-

Table 1. Toxicity tests of active fraction of garlic oil and synthetic samples to late third-instar larvae of laboratory-reared *Culex pipiens quinquefasciatus* Say.

Compounds used	Mean percentage of mortality at indicated treatment concentrations (ppm)*							
	1	3	5	7	10	50	100	200
Natural sample†	3	64	100	100	100			
Synthetic mixture of diallyl di- and trisulfides	4	76	100	100	100			
Diallyl disulfide	4	70	100	100	100			
Diallyl trisulfide	0	49	92	100	100			
Diallyl sulfide	0	0	0	0	0	0	0	16
Dipropyl disulfide and dipropyl trisulfide	0	0	0	0	0	0	0	0

* Each mean based on five replications; 50 larvae per replicate; mortality scored after 24 hours.
† The ratio of diallyl di- and trisulfide varies with the variety of garlic used.

poses for a long time. This, together with the wide range of activity of the oil, suggests that garlic oil or its active principle, whether natural or synthetic, could be used as pesticides.

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Hypertension of Renal Origin: Evidence for Two Different Mechanisms

Abstract. *Antibody to angiotensin II, or a specific peptide competitive inhibitor of angiotensin II, was used to investigate the role of the renin-angiotensin system in two types of renal hypertension in rats. The data indicate that angiotensin II is in fact critically involved in the pathogenesis of the form of renal hypertension in which one renal artery is clamped and the contralateral kidney is left in place, but that it probably plays no significant role in the maintenance of experimental renal hypertension in which the opposite kidney has been removed.*

Goldblatt and co-workers first induced chronic arterial hypertension in dogs by partially occluding one renal artery and removing the opposite kidney (*1*). Subsequently, increased renin secretion by such clipped kidneys was demonstrated (*2*). Renin acts enzymatically to release angiotensin I from a plasma globulin (*3*). Angiotensin I is then converted by other enzymes to the active octapeptide angiotensin II, the most potent pressor substance known. Accordingly, it has seemed reasonable to assume that increased plasma angiotensin II is the cause of experimental renal hypertension and its various naturally occurring counterparts in man. However, numerous studies have failed to establish this assumption as fact, and both plasma renin and angiotensin II levels are often normal in chronic renal hypertension of various species, including man (*4*). Furthermore, in other studies in which antibodies to renin or angiotensin were either administered or induced it very often has not been possible to correct this type of hypertension (*5, 6*). Therefore, the etiologic role of angiotensin II in causing or maintaining renal hypertension is open to question.

In our experiments reported here, angiotensin II antibodies were administered intravenously to rats with chronic renal hypertension, and the effects were compared with the administration of a new highly specific synthetic peptide competitive inhibitor of angiotensin II. This compound, [sarcosine^1–Ala^8]angiotensin II, completely blocks the pressor action of exogenous angiotensin II in rats and dogs when given in approximately equimolar amounts (*7*). The compound itself has no pressor or depressor activity when given intravenously. Its biological half-life is approximately 12 minutes. The use of a similar peptide inhibitor of angiotensin II has been described (*8*).

Two types of renal hypertension were studied. In the first type a silver clip was placed on the left renal artery and the other kidney was left untouched (this is referred to as two-kidney Goldblatt hypertension). In the second, a silver clip was placed on the left renal artery, and the contralateral kidney was removed (one-kidney Goldblatt hypertension). The two groups of hypertensive animals, together with an additional control group, were maintained for 6 weeks on Purina rat chow (0.42 percent sodium content) and allowed free access to water. All animals weighed 350 to 450 g. A mean blood pressure of 121.3 ± 6.6 mm-Hg (mean ± standard error) was found in normal rats. The mean blood pressure of two-kidney hypertensive animals was 195.6 ± 10.8 mm-Hg. The difference in blood pressures between the two hypertensive groups was not statistically significant.

Antibodies to angiotensin II were prepared in rabbits (*9*). The apparent affinity constant of the antibody was calculated to be 3×10^{11} liter/mole.

The animals were anesthetized with intraperitoneal pentobarbital (5 mg/100 g). Both jugular veins were cannulated (PE-10 catheter) for injection or infusion, and the blood pressure was continuously monitored with a strain gauge through a carotid artery catheter (PE-50). During an initial control period standard doses of angiotensin II (50 ng) and of norepinephrine (100 ng) were injected through the cannula; pooled rabbit serum was then injected as a control, and the animals were challenged with angiotensin II and norepinephrine standards. Then, either undiluted serum containing angiotensin II antibody (0.3 or 0.6 ml) was injected as a single dose or, alternatively, the angiotensin II inhibitor was infused at a rate of 4 μg/min for 1 hour. After the antibody injection or during infusion of the inhibitor, the pressor effects of the standard amounts of exogenous angiotensin II and norepinephrine were checked periodically.

The blood pressure response to exogenous angiotensin II in normal rats ($n = 10$) was blocked by as little as 0.3 ml of antibody. The amount of antibody also induced an immediate fall in blood pressure of 47.5 ± 2.5 mm-Hg, which was sustained for about 5 minutes, before it gradually returned to baseline levels (in the next 10 minutes). However, the pressor effects of exogenous angiotensin II remained completely blocked for up to 3 hours. Blood pressure response to exogenous norepinephrine was never affected by the administration of the antibody. Administration of the pooled rabbit serum had no effect on the blood pressure or the pressor activity of angiotensin II or norepinephrine.

Twice as much antibody (0.6 ml) was required to abolish the pressor effect of exogenous angiotensin II in the two-kidney hypertensive rats ($n = 6$) and in the one-kidney rats ($n = 6$). However, in the two-kidney animals antibody administration induced a fall in mean blood pressure of 41.0 ± 4.0 mm-Hg ($P < .001$), whereas in the one-kidney rats the blood pressure fell by only 10.0 ± 6.4 mm-Hg, and this change was not significant ($P > .1$) (Fig. 1). The reduction in blood pressure lasted for an average of 16 min-