also possible with the use of nystatin counterselection and by substituting a nonfermentable carbon source (glycerol) for glucose, along with a few modifications as follows. (i) Cells were grown for 48 hours on yeast extract and peptone (YEP) plus 5 percent glucose before EMS treatment in order to reduce the number of mitochondria per cell. These glucose-repressed conditions result in cytological changes similar to those seen with anaerobic growth, namely, a reduction from 15 or 20 mitochondria in a respiring cell to two or three mitochondria per repressed cell. (ii) The cells were returned to YEP plus 5 percent glucose after treatment with ethylmethanesulfonate to enhance the segregation of a single mitochondrial population of a sensitive mutant. (iii) After nitrogen starvation, the cells were grown on petite medium plus 1 mg of neomycin per milliliter before addition of nystatin. (iv) Finally the cells were grown on petite medium for 24 hours after the nystatin was washed away. One of the ten mutants isolated is unable to grow on petite medium containing 800 μg of neomycin per milliliter, but grows well on petite medium without neomycin and on YEPD plus 3 mg of neomycin per milliliter. This indicates that some mitochondrial function is impaired by the addition of neomycin. We have been unable to analyze this mutant genetically because the ascospores do not germinate.

Strain AA-89, which was selected for streptomycin sensitivity by suppression with low concentrations of streptomycin and which is also cold-sensitive (allelic with streptomycin sensitivity), has an altered ribosomal profile (Fig. 1) when grown at the nonpermissive temperature (15°C); thus it shows the cold-sensitive phenotype defective in subunit assembly similar to those reported for bacteria (*17*). The cytoplasmic ribosomes isolated from this mutant when grown at the permissive temperature (30°C) are sensitive to neomycin and streptomycin in an in vitro protein-synthesizing system (*13*). Our results indicate that AA-89 is a ribosomal mutant in the sense of Apirion (*18*), and we conclude that the streptomycin and neomycin sensitivity of the strain results from this alteration in ribosome structure.

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Environmental Nitroso Compounds: Reaction of Nitrite with Creatine and Creatinine

Abstract. *Creatine reacts with nitrite under acid conditions to produce first sarcosine and then* N*-nitrososarcosine, which is a weak carcinogen in the rat. Creatinine reacts with acidified nitrite to produce either creatinine-5-oxime or 1-methylhydantoin-5-oxime, depending on reaction conditions. The toxicity and environmental significance of these compounds is not yet known.*

The carcinogenicity of large numbers of nitrosamines has been demonstrated in various tissues of many animal species (*1*). It is possible that nitrosamines will form in the environment whenever nitrite and secondary or tertiary amines occur together. Certain foodstuffs have already been shown to contain simple alkyl nitrosamines (*2*), and reaction of nitrite and various secondary amines in human gastric juice at *p*H 1.5 in vitro also yielded nitrosamines (*3*). These compounds might represent a health hazard (*4*), and their possible relevance to the etiology of human cancer has been reviewed (*1, 5, 6*).

Nitrates and nitrites are frequently used as preservatives and for color fixation in foods. Extensive use of nitrates as fertilizers can lead to high accumulated levels in water supplies and plant tissue. Microorganisms, particularly the coliform group or *Clostridia*, reduce nitrate to nitrite; thus nitrite can accumulate during the storage of fodder crops and during the storage and processing of vegetables and food to which nitrate has been added.

Creatine, present in muscular tissue of many vertebrates, is a normal constituent of meat. A large portion of creatine in muscle is combined with phosphoric acid as phosphocreatine, but this compound is readily hydrolyzed to creatine and creatinine in acid solution (*7*). Creatinine, the end product of creatine metabolism, is found together with creatine in muscle tissues, milk, and blood. Other significant sources of creatinine include grain seeds and other vegetable matter, certain fish, and crab meat extract (*8*).

In view of the abundance of nitrite, creatine, and creatinine in our environment, and because these compounds are frequently ingested simultaneously in a normal diet, we thought that it was important to investigate their mutual reactivity.

We first demonstrated the formation of *N*-nitrososarcosine by reaction of creatine with nitrite: *N*-nitrososarcosine has been shown to induce cancer of the esophagus in the rat (*6*). To a stirred solution of 5.2 g of creatine (Nutritional Biochemicals) dissolved in 15 ml

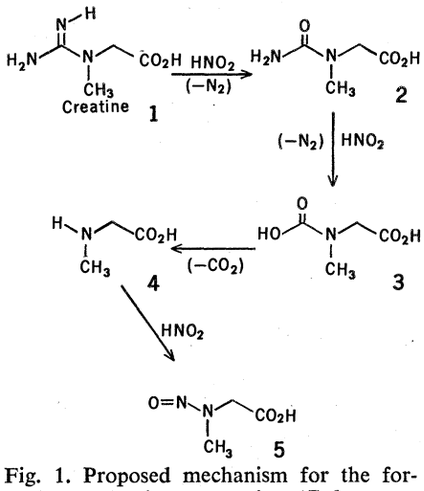

Fig. 1. Proposed mechanism for the formation of *N*-nitrososarcosine (**5**) by reaction of creatine and nitrite.

of 25 percent HCl, 5.3 g of $NaNO_2$ in 20 ml of distilled water was added dropwise. After standing at 25°C for 2½ hours, the reaction mixture was saturated with NaCl and extracted four times with 10-ml portions of diethyl ether. The extracts were combined and then dried over anhydrous magnesium sulfate. Removal of the solvent under reduced pressure yielded 1.1 g of a yellow oil. The oil was purified by elution from a silica gel column with diethyl ether, and yielded 1.07 g of *N*-nitrososarcosine that was characterized by its identity to authentic material (*9*) synthesized directly from sarcosine.

A possible mechanism for this reaction (Fig. 1) involves reaction of creatine (**1**) with 1 mole of nitrous acid to yield *N*-carbamyl-*N*-methylglycine (**2**) as the first step. This product can then react with more nitrous acid to yield *N*-carboxyl-*N*-methylglycine (**3**), which decarboxylates to give *N*-methylglycine

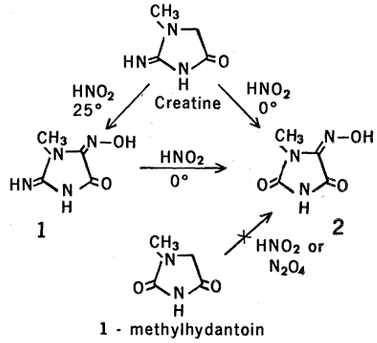

Fig. 2. Formation of creatinine-1-oxime (**1**) and 1-methylhydantoin-5-oxime (**2**) by reaction of creatinine and nitrite.

(sarcosine, **4**). A final nitrosation yields *N*-nitrososarcosine (**5**).

Nitrosation of creatinine has been examined by Schmidt (*10*), but the products have never been characterized unambiguously.

To a stirred solution of 6.1 g of creatinine (Nutritional Biochemicals) dissolved in 25 ml of 10 percent HCl we added dropwise, over a period of 10 minutes, 26.3 g of $NaNO_2$ in 30 ml distilled water. The solution became hot and turned green and then orange, and a yellow solid precipitated. Stirring was continued for 1 hour; the solid was removed by filtration and washed with water. Recrystallization of the solid from hot water, after treatment with animal charcoal yielded 2.2 g of a white solid, m.p. 255°C (with decomposition), which gave negative results in a Liebermann test for a nitroso group. The compound was identified by analysis and spectroscopic methods (*11*) as creatinine-5-oxime (**1**; Fig. 2).

When the reaction described above was carried out at 0°C, the solution turned orange; a pink solid precipitated which, after 1 hour, was collected, washed, and dissolved in hot water. The hot water solution was treated with animal charcoal, filtered, and subsequently yielded 1.75 g of white crystals, melting at 181°C. This compound also gave a negative Liebermann test and was identified by analysis and spectroscopic methods (*12*) as 1-methylhydantoin-5-oxime (**2**; Fig. 2).

Creatinine-5-oxime was readily converted to 1-methylhydantoin-5-oxime. One gram of the crude yellow creatinine-5-oxime was suspended in 10 ml of 10 percent HCl and cooled to 0°C. Over a period of 5 minutes, 4 g of $NaNO_2$ in 10 ml of distilled water was added to the stirred suspension. Stirring was continued for 3 hours at 0°C, and the slurry gradually turned pink. Recrystallization of the pink solid from water after treatment with animal charcoal gave 0.35 g of 1-methylhydantoin-5-oxime.

An attempted synthesis of 1-methylhydantoin-5-oxime from the parent 1-methylhydantoin (Aldrich Chemical Co., Inc., Milwaukee, Wis.) by reaction with excess sodium nitrite in acid solution failed to yield any product at either 0° or 60°C. Direct reaction with nitrogen tetroxide in acetic acid (*13*) also yielded no product.

These reactions are summarized in Fig. 2. Understanding of the mechanisms of the reactions remains obscure, although the imino group in creatinine does seem implicated in its reactivity. The toxicities of creatinine-5-oxime and 1-methylhydantoin-5-oxime are as yet unknown.

We have demonstrated in vitro the formation of a known weak carcinogen for the rat, *N*-nitrososarcosine, and two oximes of undetermined toxicity, from precursors in abundant supply in man's environment. Since the relative proportion of creatine and creatinine in any system will be a function of temperature and *p*H (*7, 14*), the amounts of various end products formed by reaction with nitrite will vary from system to system. It remains to be determined whether these reactions actually take place in foods or in the mammalian stomach, and their significance in the incidence of human cancer must be evaluated.

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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.