9114

Bactericidal Properties of Acrolein.*

RICHARD E. VOLLRATH, LUCILE WALTON AND CARL C. LINDEGREN.
From the University of Southern California, Los Angeles.

Walker, Lindegren, and Bachmann,[1] Walton, Herbold, and Lindegren,[2] McKnight and Lindegren,[3] have shown that the vapors escaping from freshly crushed garlic, as well as those from onions are extremely active bactericides. Minchin[4] has presented clinical evidence that garlic and onion have therapeutic value, especially in the treatment of tuberculosis. The present paper deals with the problem of identifying the active agent in garlic. The allyl polysulfides and their relatives, which give garlic its characteristic odor were found by the writers to have negligible bactericidal action. Since aldehydes are known to be present in garlic, it appeared reasonable to try the effect of acrolein (allyl aldehyde). Acrolein was investigated and found to be powerfully bactericidal. All the evidence presented supports the view that acrolein, or possibly some related unsaturated aldehyde is the active bactericide of garlic.

Koch and Fuchs[5] investigated the use of acrolein as an antiseptic, but made no further reference to possible therapeutic application.

There are two possible methods of identifying the bactericidal substance in garlic. One is to separate the complex mixture into its components and to test the bactericidal effects of each. This method appeared more difficult than the following alternative. A series of compounds were prepared on the basis of these clues:

(1) Garlic contains allyl disulfide and related polysulfides. These are the oils giving garlic its characteristic odor.

(2) Garlic and particularly onions contain substances which blister the skin and provoke tears.

(3) The bactericidal substance is volatile, indicating a compound of low molecular weight and simple structure.

The following compounds were synthesized and purified by dis-

* The writers are indebted to Mr. A. K. Humphries, President of the Vitab Products, Inc., San Francisco, for financial assistance.

1 Walker, J. C., Lindegren, C. C., and Bachmann, F. M., *J. Agr. Res.*, 1925, **30**, 175.

2 Walton, L., Herbold, M., and Lindegren, C. C., *Food Res.*, **1**, 163.

3 McKnight, R. S., and Lindegren, C. C., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 477.

4 Minchin, W. C., Bailliere, Tindall, and Cox, London, 1927.

5 Koch, E., and Fuchs, G., *Zentralblatt für Bakteriologie und Parasitenkunde*, 1899, **26**, 560.

tillation at the boiling point characteristic of each: methyl sulfide, ethyl sulfide, ethyl disulfide and allyl disulfide. A mixture of allyl polysulfides up to the pentasulfide was prepared. A portion of this was distilled *in vacuo*. A portion was washed with water in a separatory funnel and used without distillation. The purpose of this was to avoid the possibility of decomposing the higher polysulfides. Allyl isothiocyanate was also prepared.

Alcohols and aldehydes are known to occur in plants and since there are allyl compounds in garlic, it was thought worth while to investigate allyl alcohol and allyl aldehyde (acrolein).

When an agar plate is exposed to the vapors from one gram of freshly crushed garlic for 30 minutes, a sufficient amount of volatile bactericide is taken up by the agar to kill all the bacteria in a heavy suspension of *E. coli* streaked on the surface immediately after exposure. After the plate has been streaked, it is incubated without further exposure to the vapor.

Thirty-minute exposure of a plate to one gram of crushed garlic sterilizes the plate. Furthermore, not more than 10% of any of these compounds could be present in garlic. For these reasons, if 0.1 gm. of a compound does not sterilize the agar surface, it cannot be the active substance in garlic.

Each of the compounds was tested separately, to determine bactericidal effect of direct contact of organism and compound. On the agar surface of one plate was placed 1.0 cc. of the compound. To this was added 0.1 cc. of a heavy suspension of *E. coli* and the mixture was spread over the agar with a glass needle. In a similar manner on a second plate 0.5 cc. of compound and 0.1 cc. of *E. coli* was spread. On a third plate 0.1 cc. of compound and 0.1 cc. of *E. coli* were spread. The plates were then incubated at 37.5°C. for 24 hours. This served to measure the bactericidal activity of the compound in direct contact with the organism.

In a second series, each of the compounds was tested to determine the bactericidal effect of its vapor. A piece of filter paper was placed in a petri-dish cover and moistened with one cc. of the compound. Over this was inverted the petri-dish bottom containing the solidified agar. After exposure for 30 minutes to the vapor of the compound at 37.5°C. the agar was streaked with a heavy suspension of *E. coli*. The cover was then replaced by a clean one and the plate incubated for 24 hours at 37.5°C. The same test was made with 0.5 cc. and 0.1 cc. All the compounds were bactericidal when placed directly on the plates; however, allyl aldehyde was the only compound whose vapors showed an activity comparable with that of the bactericidal substance in garlic.

Bactericidal Activity of Acrolein. Six dilutions of acrolein in water were made ranging from 1:100 to 1:10,000,000. To each member of a duplicate series was added one percent by volume of sterile liquid white of egg (not a solution of dried egg albumin),

TABLE I.
Bactericidal Effects of Acrolein Solutions.

	Albumin absent				Albumin present			
Time, hr.	6	12	24	48	6	12	24	48
E. Coli								
Conc.								
1/100	0	0	0	0	0	0	0	0
1/1,000	0	0	0	0	0	0	0	0
1/10,000	1	0	0	0	1	1	0	0
1/100,000	3	2	0	0	4	4	3	4
1/1,000,000	4	3	2	0	4	4	3	4
1/10,000,000	4	3	2	0	4	4	4	4
Control	4	4	4	4	4	4	4	4
B. Subtilis								
1/100	4	2	0	0	4	4	4	3
1/1,000	4	4	2	4	4	4	4	4
1/10,000	4	4	4	4	4	4	4	4
1/100,000	4	4	4	4	4	4	4	4
1/1,000,000	4	4	4	4	4	4	4	4
1/10,000,000	4	4	4	4	4	4	4	4
Control	4	4	4	4	4	4	4	4

and the mixtures were allowed to stand for 24 hours. Agar plates were exposed for 30 minutes to the vapors from one cc. of each of these solutions placed on paper as described before. These plates were then streaked with *B. subtilis.* A duplicate run was made with *E. coli.* Fresh covers were put on and the dishes were incubated at 37.5°C. for 24 hours. The plates exposed to concentrations of 1:100 were sterile both with or without egg-white. All others showed full or nearly full growth. The vapor pressure of the acrolein above the 1:100 solution was calculated as being 0.6 mm. of mercury. This is approximately one part of gaseous acrolein to 1000 parts of air. The activity of 1 gm. of a 1:100 solution of acrolein is of the same order as the activity of 1 gm. of garlic.

Tests were made to determine the bactericidal activity of aqueous acrolein-solutions upon bacteria immersed in the solution. One-tenth cc. of a heavy suspension of *E. coli* was placed in 10 cc. of each of the serial dilutions. This series was duplicated using a heavy suspension of *B. subtilis.* After 6, 12, 24, and 48 hours, agar plates were streaked and incubated.

The number 4 in Table I indicates growth equivalent to that of the control; zero indicates no growth. The figures 1, 2, and 3 indicate intermediate degrees of growth.

Lewin[6] showed that (1) acrolein is lethal to mammals only in large amounts (0.25 gm. per kilo), (2) acrolein-vapor appears in the breath shortly after subcutaneous injection, (3) the symptoms of acrolein-poisoning in man are dizziness, nausea, and diarrhea. These symptoms have been observed in our laboratory in subjects who have eaten several ounces of garlic. The fact that detectable amounts of acrolein appear in the breath after injection of acrolein suggest its possible value as a disinfectant of the respiratory tract. Since it is lethal only in large amounts and bactericidal in small amounts, we propose to investigate its therapeutic possibilities further.

Summary. The well known sulfides responsible for the peculiar odor of garlic are not responsible for its bactericidal activity. Acrolein was found to be a highly active bactericide. Its properties are such that it gives promise of being a respiratory disinfectant. Its general properties suggest that it or related compounds may be the bactericide of garlic.†

The writers are grateful to Dr. Henry Borsook for his encouraging advice.

9115 P

Acidosis Associated with the Administration of Para-amino-benzene-sulfonamide (Prontylin).

HAMILTON SOUTHWORTH. (Introduced by P. H. Long.) (With the technical assistance of Florence White.)

From the Johns Hopkins Hospital, Baltimore, Md.

In the last 3 months at Johns Hopkins Hospital about 50 cases have been treated with para-amino-benzene-sulfonamide (Prontylin) in doses of 0.04 to 0.12 gm. per kilo per day. Two of these cases have shown clinical acidosis.

Case A. G. J., 29 yrs., colored female, was admitted for an acute beta hemolytic streptococcal tonsillitis. Prontylin was started by mouth and her temperature fell to normal in 36 hours. After 48 hours, however, she began definitely overbreathing and the CO_2 combining power of her plasma was 36.2 vol. %. At this point

[6] Lewin, L., *Arch. Exp. Path. Pharm.*, 1900, **43**, 1351.

† Our recent results show that acrolein is much more poisonous than Lewin found. Proof that acrolein, croton aldehyde, or a similar substance is present in garlic has been obtained by a color reaction.