inapparent (subclinical) infection. This phase of biochemical activity is associated with or followed by immune phenomena by which the guinea pig acquires active resistance to subsequent infection.

The present study still in progress extends the previously observed effectiveness of p-aminobenzoic acid in other, milder rickettsioses[4, 5] and proves its protective value in the highly virulent spotted fever.

LUDWIK ANIGSTEIN
MADERO N. BADER

UNIVERSITY OF TEXAS
SCHOOL OF MEDICINE,
GALVESTON

STUDIES ON THE MECHANISM OF ANTIBACTERIAL ACTION OF 2-METHYL-1,4-NAPHTHOQUINONE[1]

THE antibacterial activity of quinones has been recognized since 1911.[2, 3, 4, 5, 6, 7] Interest in this phase of their activity has been stimulated in recent years by the discovery of the quinone structure in antibiotics derived from molds.[7, 8] Synthetic 2-methyl-1,4-naphthoquinone as tested in this laboratory has been shown to be bacteriostatic and bactericidal for both gram-positive cocci and gram-negative bacilli, and a similar effect has been noted on many species of fungi. It was found that minimum effective bacteriostatic and bactericidal concentrations for *Escherichia coli* in a chemically defined medium were lower than in the usual peptone broth or agar (0.00125 per cent. to 0.0025 per cent. instead of 0.005 per cent. to 0.02 per cent.); also that in Brewer's thioglycolate medium, both bacteriostatic and bactericidal activity of concentrations as high as 0.04 per cent. were nullified in freshly prepared medium, although in medium prepared some three months previously and thoroughly shaken immediately before use, minimum effective bacteriostatic and bactericidal concentrations were approximately the same as in other peptone media. The effect of the fresh Brewer's medium did not appear to be due to its anaerobic properties, since tests on agar plates incubated in an anaerobic jar gave the same endpoints as those obtained on aerobic incubation. The following work was undertaken in an attempt to explain this behavior.

METHODS

The medium used was McCleod's synthetic medium,[9] with asparagin and $(NH_4)_2SO_4$ as sources of nitrogen, glucose, NaCl, phosphates and traces of Fe, Ca and Mg, dispensed in 8″ by 1″ test-tubes in accurately measured amounts.

The quinones employed included 2-methyl-1,4-naphthoquinone; the water-soluble sodium bisulfite addition product of 2-methyl-1,4-naphthoquinone; 2-methyl-3-methoxy-1,4-naphthoquinone, 2-methyl-3-chloro-1,4-naphthoquinone and 2,6-dimethyl-1,4-naphthoquinone, all pure synthetic compounds prepared in the chemistry research laboratories of this corporation. Solutions were made up aseptically in acetone or water, depending on their solubilities, in concentrations 25 times as strong as the highest final concentration desired in the medium. Serial dilutions were made by halves and were added in 1 ml amounts to 24 ml of sterilized medium (to 23 ml if a substance to be tested for antagonism of antibacterial action was also to be added). Substances to be tested for antagonism of antibacterial action were prepared in sterile aqueous solution and 1 ml of a standard dilution was added to each culture tube of a series containing varying concentrations of the quinone. Acetone and water controls were included in each series. The inoculum of *Escherichia coli* was 0.1 ml of a 24-hour culture in the synthetic medium diluted 1:4 (approximately 20 to 25 million bacteria, or 800,000 to 1 million per ml of culture). Incubation was at 37° C. except when the volatile ethyl mercaptan was used, when both mercaptan and quinone control series were incubated at 30° C. Readings for bacteriostasis were made daily for 4 to 5 days by observations of visual turbidity. Daily subcultures were made by streaking each culture on an agar plate to determine whether organisms were killed or merely kept from growing.

Nitroprusside tests for sulfhydryl groups were made on mixtures of solutions of quinones and (a) thioglycolic acid neutralized with sodium carbonate and (b) cysteine hydrochloride by adding 3 N NH_3 and a dilute solution of sodium nitroprusside.

RESULTS

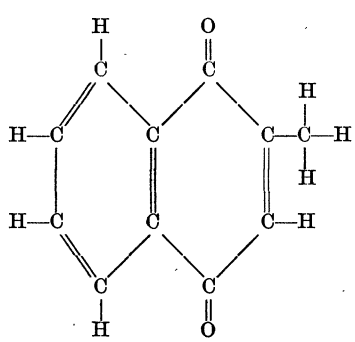
Thioglycolic acid neutralized with sodium carbonate and sodium thioglycolate (Eastman) in molar concentrations greater than 2-methyl-1,4-naphthoquinone suppressed both bacteriostatic and bactericidal activity of the quinone on *E. coli* in synthetic medium. This effect might have been due either to their action as reducing agents or to the sulfhydryl groups; consequently other reducing agents were tested. Sodium bisulfite and sodium hydrosulfite partially antagonized

[1] A preliminary report.
[2] W. Thalhimer and B. Palmer, *Jour. Infect. Dis.*, 9: 181, 1911.
[3] E. A. Cooper, *Biochem. Jour.*, 6: 362, 1912.
[4] G. T. Morgan and E. A. Cooper, *Biochem. Jour.*, 15: 587, 1921.
[5] G. T. Morgan and E. A. Cooper, *Soc. Chem. and Ind.*, 43: 352, 1924.
[6] W. D. Armstrong, W. W. Spink and J. Kahnke, *Proc. Soc. Exp. Biol. and Med.*, 53: 230, 1943.
[7] A. E. Oxford and H. Raistrick, *Chem. and Ind.*, 61: 128, 1942.
[8] S. A. Waksman and H. B. Woodruff, *Jour. Bact.*, 44: 373, 1942.
[9] Colin McCleod, *Jour. Exp. Med.*, 72: 217, 1940.

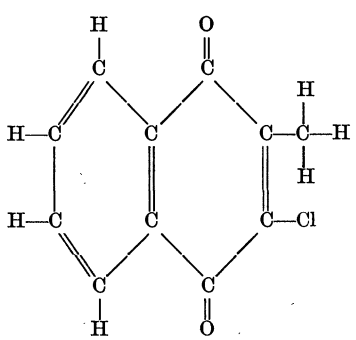
the bacteriostatic and bactericidal effects of the quinone, while stannous chloride, potassium formate and sodium thiosulfate had no effect. These results pointed to the probable responsibility of the sulfhydryl group for antagonism noted with thioglycolate; consequently ethyl mercaptan and cysteine hydrochloride were tested. Suppression of anticoli activity of 2-methyl-1,4-naphthoquinone was again noted with these two sulfhydryl compounds.

Sodium thioglycolate and cysteine hydrochloride also antagonized the anticoli activity of 2-methyl-3-chloro-1,4-naphthoquinone, with –Cl instead of –H in the 3 position on the quinone ring, and that of 2,6-dimethyl-1,4-naphthoquinone. However, these sulfhydryl compounds were without effect on the anticoli action of 2-methyl-3-methoxy-1,4-naphthoquinone with the $-OCH_3$ group instead of –H or –Cl in the 3 position.

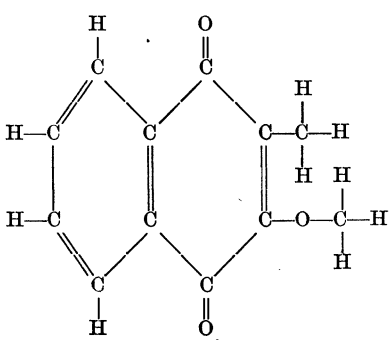
Nitroprusside tests were difficult to read because of the interfering color of the quinone which masked weakly positive tests. A colored precipitate resulted from the mixture of cysteine and 2-methyl-1,4-naphthoquinone, but not from the mixture of cysteine and the methoxy quinone. As nearly as could be determined under these circumstances, 2-methyl-1,4-naphthoquinone and 2-methyl-3-chloro-1,4-naphthoquinone in excess on a molar basis eliminated the color due to —SH groups in these tests, while 2-methyl-3-methoxy-1,4-naphthoquinone did not, although a slightly weaker color reaction was elicited with the latter compound in excess than in control tests with the same concentration of sulfhydryl.



2-methyl-
1,4-naphthoquinone



2-methyl-3-chloro-
1,4-naphthoquinone



2-methyl-3-methoxy-
1,4-naphthoquinone

DISCUSSION

Interference of sulfhydryl compounds with the antibacterial properties of mercury antiseptics was reported by Fildes in 1940,[10] and a theory of their mode of action was proposed. Nungester, Hood and Warren[11] suggested a practical application of this finding in testing disinfectants of this nature. Thioglycolate, glutathione and cysteine have been found antagonistic to a variety of antibiotic substances including penicidin,[12] penicillin,[13,14] citrinin, gliotoxin, pyocyanin and several from plants,[13] clavicin and penicillic acid.[13,15] Cavallito and Bailey[13] believe that the main mode of action of many antibiotic substances lies in their ability to interfere with the normal function of sulfhydryl groups in bacterial metabolism.

More recently, Geiger and Conn[15] have suggested that since clavicin and penicillic acid are both α, β-unsaturated ketones having as their only common structural detail the —CH=C—C=O group, the latter grouping is concerned with their antibacterial potencies. The inactivation of both of these antibiotics, as well as some synthetic, α, β-unsaturated ketones, by sulfhydryl compounds led them to suggest that antibiotic properties of these substances are due to their reaction with sulfhydryl groups essential to the activity of bacterial enzyme systems, or with sulfhydryl-containing metabolites essential to bacteria.

2-methyl-1,4-naphthoquinone may also be considered an α, β-unsaturated ketone, the antibacterial activity of which is suppressed by –SH compounds. Its mode of antibacterial action therefore appears to be similar to that suggested by several groups of investigators for antibiotic substances, some of which are known to contain quinone structures and some not. The fact that 2-methyl-3-chloro-1,4-naphthoquinone and 2,6-dimethyl-1,4-naphthoquinone with easily replaceable –Cl and –H in the 3 positions on the quinone rings, are also inactivated by sulfhydryl compounds,

[10] Paul Fildes, *Brit. Jour. Exper. Path.*, 21: 67, 1940.

[11] W. J. Nungester, M. N. Hood and M. K. Warren, *Proc. Soc. Exp. Biol. and Med.*, 52: 287, 1943.

[12] N. Atkinson and N. Stanley, *Austral. Jour. Exp. Biol. and Med. Sci.*, 21: 249, 1943.

[13] C. J. Cavallito and J. H. Bailey, SCIENCE, 100: 390, 1944.

[14] R. J. Hickey, SCIENCE, 101: 232, 1945.

[15] W. B. Geiger and J. E. Conn, *Jour. Amer. Chem. Soc.*, 67: 112, 1945.

while 2-methyl-3-methoxy-1,4-naphthoquinone with the $-OCH_3$ group in that position is not, lends support to the hypothesis that the 3 position on the quinone ring is important in the inhibition of bacterial growth by these compounds. The antibacterial activity of the methoxy quinone, however, even in the presence of sulfhydryl compounds, suggests that the foregoing may be only one of the mechanisms involved.

Details of these studies will be reported in a subsequent communication.

SUMMARY

Thioglycolic acid neutralized with sodium carbonate, sodium thioglycolate (Eastman), ethyl mercaptan, cysteine hydrochloride and certain sulfur-containing reducing agents (sodium bisulfite and sodium hydrosulfite) antagonize the antibacterial action of 2-methyl-1,4-naphthoquinone on *Escherichia coli* in a synthetic medium. Other reducing agents such as stannous chloride, potassium formate and sodium thiosulfate, show no such antagonism. The antibacterial activities of 2-methyl-3-chloro-1,4-naphthoquinone and 2,6-dimethyl-1,4-naphthoquinone are also abolished by excess thioglycolate and cysteine, while that of 2-methyl-3-methoxy-1,4-naphthoquinone with $-OCH_3$ instead of –Cl or –H in the 3 position on the quinone ring, is not. These findings suggest that the mode of antibacterial action of 2-methyl-1,4-naphthoquinone is by blocking essential enzymes through combination with sulfhydryl groups, or through combination with sulfhydryl groups of essential bacterial metabolites. This combination may take place in the 3-position on the quinone ring. This mode of action is similar to that suggested by other investigators for several antibiotic agents including penicillin. The antibacterial activity of the methoxy quinone, however, even in the presence of sulfhydryl groups, suggests that the foregoing explanation may not be the complete one.

CHARLOTTE A. COLWELL
MARY MCCALL

MICROBIOLOGY LABORATORIES,
VELSICOL CORPORATION,
CHICAGO, ILLINOIS

SCIENTIFIC APPARATUS AND LABORATORY METHODS

ELECTRON SHADOW MICROGRAPHY OF THE TOBACCO MOSAIC VIRUS PROTEIN[1,2]

WE have recently shown[3] that electron micrographs of tobacco mosaic and influenza viruses shadowed by the oblique deposition of a very thin evaporated metal film reveal many details that have not otherwise been seen. For this earlier work the evaporated film was chromium in a calculated thickness of *ca.* 80A. This procedure is satisfactory as long as one is concerned with objects like bacteria[4] and most viruses that are large compared with the thickness of the metallic layer deposited on them during shadowing. In attempts to detect very small particles it has been found that they may be recorded, though smaller in diameter than the thickness of chromium they accumulate when shadowed. Though this does not in itself interfere with their detection, it gives an obviously false impression of shape and renders difficult and inaccurate any measurements of their true size. We have accordingly sought a metal of higher electronic scattering power than chromium which would give continuous films upon evaporation and could therefore be used in much thinner layers for shadowing. Gold in a calculated thickness of 5 to 10A meets these requirements. It replaces chromium to great advantage in the photography of the smallest viruses and of other particles of macromolecular size.

An electron micrograph, made with an RCA Type EMB instrument, of a gold-shadowed tobacco mosaic virus preparation[5] is shown in Fig. 1. Compared with our previously published picture of this substance[3] there is a clear improvement in the delineation of the tobacco mosaic fibrils, though it should be emphasized that when allowance is made for the different thicknesses of the coating metals the same general idea of fibril-shape emerges from a consideration of each photograph. There is also a reduction in the texture of the background detail which has its origin in the ultimate structure of the collodion substrate. This reduction is important for work with other macromolecules because such molecules can be studied on collodion substrates only as long as a discrimination can be made on the photographs between the macromolecules and the structural details of collodion.

Since, as the figure indicates, collodion has an ultimate structure approaching in dimensions the width of the tobacco mosaic virus molecule and since far smaller particles can be recognized through shadow-casting, it has been important to find and utilize a

[1] From the Department of Physics and the Virus Laboratory, Department of Epidemiology, School of Public Health, University of Michigan.

[2] Supported in part by a grant from the National Foundation for Infantile Paralysis, Inc.

[3] R. C. Williams and R. W. G. Wyckoff, *Proc. Soc. Exp. Biol. and Med.*, 58: 265, 1945.

[4] R. C. Williams and R. W. G. Wyckoff. In press.

[5] We are indebted to W. M. Stanley for the purified tobacco mosaic virus protein used in this work.