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Antibacterial Effects of Quinones.*

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Fosdick, Fancher and Calandra[1] reported that small amounts of synthetic vitamin K (2-methyl-1,4-naphthoquinone) prevented the production of acid when saliva-glucose mixtures were incubated. Armstrong and Knutson[2] demonstrated that this effect of 2-methyl-1,4-naphthoquinone is due to the quinone structure of the compound and found that the same result could be obtained, in varying degree, with other naphtho-, tolu- and benzoquinones. Increased interest in the bacteriostatic and bactericidal influence of quinones is derived from the reports relating the activity of at least two antibiotic substances to quinones. Fumigatin (3-hydroxy,-4-methoxy,2:5-tolu-quinone) produced by *Aspergillus fumigatis,* possesses a definite ability to inhibit the multiplication of several gram-positive organisms.[3] Certain synthetic dimethyoxy quinones related structurally to fumigatin have been found to exert a remarkable antibacterial effect *in vitro*.[4,5] Waksman and Woodruff[6] note that actinomycin contains a quinone group and report a comparison of the bacteriostatic effect of tolu-p-quinone with that of several antibiotic agents. The same quinone was found[7] to exhibit a high degree of effectiveness in interfering with the luminescence of *Photobacterium fischeri* and a considerable bacteriostatic action against *Streptococcus pyogenes.*

The previous report[2] from this laboratory noted that several of the quinones which are effective in the prevention of acid formation in incubated saliva-glucose mixtures had been found to inhibit the growth of certain strains of streptococcus, staphylococcus and pneumococcus in liquid media. The present report presents quantitative data with respect to the required bacteriostatic and lethal concentrations of several quinones against two species of gram-positive pathogenic cocci. The coagulase-positive strain of staphylococcus was originally isolated from a patient with an osteomyelitic sinus. This strain was transferred daily in broth and was carried through at least 3 successive transfers before being employed in these experiments. After 14 transfers the culture was discarded and a fresh series of transfers were started from a colony on an agar plate. The streptococcus was strain C703 kindly supplied by Dr. Eleanor Bliss of the Johns Hopkins University Hospital. This strain was transferred twice daily in glucose broth and several times during the progress of the work it was reisolated from blood agar plates.

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1 Fosdick, L. S., Fancher, O. E., and Calandra, J. C., *Science,* 1942, **96**, 45.

2 Armstrong, W. D., and Knutson, J. W., Proc. Soc. Exp. Biol. and Med., 1943, **52**, 307.

3 Oxford, A. E., and Raistrick, H., *Chemistry and Industry,* 1942, **61**, 128.

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5 Oxford, A. E., *J. Chem. Soc.,* 1942, p. 577.

6 Waksman, S. A., and Woodruff, H. B., *J. Bact.,* 1942, **44**, 373.

7 Rake, G., Jones, H., and McKee, C. M., Proc. Soc. Exp. Biol. and Med., 1943, **52**, 136.

The quinones or other substances to be tested, which were conveniently soluble in alcohol, were made up in this solvent so that equal volumes contained equal molecular amounts of the several substances. The materials not soluble in alcohol were dissolved in sterile distilled water or an appropriate solvent† to produce solutions of the same molecular concentration as the alcoholic solutions referred to above. The required volumes (0.025 to 1.0 cc) of these solutions were transferred to sterile 25 cc volumetric flasks so as to produce in the final volume to be incubated definite molecular quantities of each substance. In the case of the experiments with the staphylococcus these quantities corresponded, on a molecular basis, to 0.125 to 5.0 mg of 2-methyl-1,4-naphthoquinone per 100 cc of the final dilution. The increment of concentration, expressed in the same manner, between flasks of each series was 0.125 mg between the first 2 flasks and 0.25 mg between subsequent flasks. The non-aqueous solvents were evaporated under reduced pressure in a vacuum desiccator causing the quinone to be deposited as a film over the wall of the flask. Sufficient water was added to all flasks so that the total volume of added water was 1.0 cc. The inoculum was prepared by adding 1.0 cc of a 10^5 dilution of a 24-hour broth culture of the staphylococcus to each 8.0 cc of a large volume of broth.‡ Nine cubic centimeters of the resulting dilution of organisms in broth were transferred to each of the 25 cc volumetric flasks, the same lot of bacterial suspension always being used to inoculate each of the flasks in a series set up to determine the bacteriostatic and lethal concentrations of a particular substance. The mouth of each flask was covered by a small sterile vial and the space between the neck of each flask and the vial was filled with a ring of cotton. The flasks were incubated with constant shaking at 37° for 4-5 hours and the incubation continued without agitation for a total of 48 hours. The solutions were examined for growth of the organisms after 24 and 48 hours of incubation by noting whether a turbidity distinct from the appearance of uninoculated broth was present. All solutions which appeared to exhibit no growth after 48 hours were tested for sterility by observing the growth, after 24 hours, on nutrient agar pour plates prepared with 1.0 cc of the solution in question. In critical cases sterility was also tested by inoculating several loopfuls of the suspected solution into 10 cc of broth.

In Table I is shown the minimum amounts of several quinones and other substances required to inhibit the growth of the staphylococcus for 24 hours and for 48 hours, and the quantities of the same substances required to kill the organisms present in the original inoculum. In several instances the results of duplicate experiments carried out on different dates are shown. In the case of a few substances, noted by the sign indicating "greater than", quantities equal to 29×10^{-6} mols per 100 cc (equivalent to 5.0 mg per 100 cc of 2-methyl-1,4-naphthoquinone) were totally ineffective against this strain of staphylococcus under the conditions of these experiments. Concentrations greater than 29×10^{-6} mols per 100 cc were not employed in this study since this amount is 10 times the quantity required to produce bacteriostatic and bactericidal results in the case of the most effective quinones.

Oxford[4] has previously reported 2,6-dimethoxy benzoquinone to inhibit the growth of a certain strain of staphylococcus in a dilution of 1:400,000 and was inferior in this regard only to 4,6-dimethoxy toluquinone. It is to be noted that 2-methyl-1,4-naphthoquinone equals, and the other naphthoquinones approach, the ability of 2,6-dimethoxy-benzoquinone to inhibit the growth or to kill the

† Water was the solvent for phenol, penicillin, and 1,2-naphthoquinone-4-potassium sulfonate; absolute alcohol for sulfathiazole; chloroform for 2,5-dichlorobenzoquinone and 2,6-dimethoxybenzoquinone; ether and alcohol for tetrachlorobenzoquinone. 2-Methyl-1-naphthol-4-amino hydrochloride and an equal quantity of sodium bisulfite were dissolved in water.

‡ The broth contained 10 g peptone (Cudahy's), 5 g extract of beef (Limco), and 5 g NaCl per 1000 cc. The final pH of various batches varied between 7.09 and 7.35. The 0.5% glucose broth had the same composition except for the addition of separately sterilized glucose and a final pH range of various batches of 7.3 to 7.6.

TABLE I.

In vitro Antibacterial Effects Against a Strain of Staphylococcus of Quinones and Other Substances Compared on a Molecular and on a Weight Basis.

Compound	No. Organisms	Minimum concentrations required for complete Inhibition of growth for 24 hr: Mols per 100 cc x10⁶	Mg per 100 cc	Inhibition of growth for 48 hr: Mols per 100 cc x10⁶	Mg per 100 cc	Sterility after 48 hr: Mols per 100 cc x10⁶	Mg per 100 cc
2-Methyl-1,4-naphthoquinone	3,200	2.9	0.50	2.9	0.50	2.90	0.50
	2,030	2.9	0.50	4.35	0.75	4.35	0.75
2,6-Dimethoxy-benzoquinone	3,580	2.9	0.49	2.9	0.49	2.90	0.49
	2,030	2.9	0.49	4.35	0.73	4.35	0.73
1,4-Naphthoquinone	4,000	7.25	1.15	8.7	1.38	8.70	1.38
1,2-Naphthoquinone	4,200	4.35	0.69	8.7	1.38	10.15	1.60
2-Methyl-1,4-naphthohydroquinone	3,600	2.9	0.50	2.9	0.50	2.90	0.50
2-Methyl-1-naphthol-4-amino hydrochloride	3,500	2.9	0.61	4.35	0.91	4.35	0.91
Benzoquinone	3,600	>29.0	>3.14	>29.0	>3.14		
	1,650	8.7	0.94	8.7	0.94		
	1,500	7.25	0.78	8.7	0.94	8.70	0.94
Hydroquinone	3,420	5.8	0.64	7.25	0.80	7.25	0.80
	1,520	5.8	0.64	7.25	0.80	7.25	0.80
Tolu-para-quinone	4,040	>29.0	>3.58	>29.0	>3.58		
	2,240	14.5	1.77	14.5	1.77		
	1,500	23.2	2.83	29.0	>3.58		
Tolu-hydroquinone	2,780	5.8	0.72	7.25	0.90	8.70	1.08
	1,750	7.25	0.90	8.7	1.08	8.70	1.08
2-Hydroxy-1,4-naphthoquinone	2,750	>29.0	>5.03	>29.0	>5.03		
1,2-Naphthoquinone-4-potassium sulfonate	1,940	>29.0	8.03	>29.0	>8.03		
2,5-Dichlorobenzoquinone	1,820	23.2	4.11	>29.0	>5.14		
Tetra-Chlorobenzoquinone	2,300	11.6	2.85	11.6	2.85	11.6	2.85
Sulfathiazole	2,900	>29.0	>7.42	>29.0	>7.42		
Phenol	1,720	>29.0	>2.73	>29.0	>2.73		

strain of staphylococcus used in these experiments. The results obtained with 2-methyl-1-naphthol-4-amino hydrochloride (Synkamin of Parke Davis) are to be explained by its ready oxidation to the corresponding 1,4-naphthoquinone, a fact which also accounts for its vitamin K activity. The marked effect produced on antibacterial ability by substitution in the quinoid ring is seen when one compares the results obtained with 1,2-naphthoquinone *versus* 1,2-naphthoquinone-4-potassium sulfonate, and those obtained with 2-methyl-1,4-naphthoquinone and 1,4-naphthoquinone *versus* 2-hydroxy-1,4-naphthoquinone. Benzoquinone and toluquinone had previously been recognized as possessing a certain degree of antibacterial ability and this study shows that the corresponding hydroquinones exhibit equal or greater effects of the same sort. In the same connection, it is to be noted that 2-methyl-1,4-naphtho-hydroquinone possesses an antibacterial potency against this strain of staphylococcus equal to that of the corresponding quinone. While a broth medium containing peptone is not the optimum medium for the demonstration of the effectiveness of sulfathiazole against staphylococci, the results with this substance and with phenol show them to be less than one-tenth as effective, under the conditions of these experiments, as the most potent of the quinones.

Table II contains the results obtained in 2 groups of experiments in which a single suspension of staphylococci in broth furnished all the inocula for the experiments in each group. All solutions in each group of experiments, one using plain broth and the other glucose broth, were incubated concurrently. It is thus possible to compare the relative bacteriostatic effectiveness of the substances named in Table II under identical experimental conditions. The data given permit the calculation that the penicillin was

TABLE II.
Comparisons of Antibacterial Effects Against a Strain of Staphylococcus Substances Under Uniform Conditions of Inoculum.

Compound	Minimum concentrations required for complete: Inhibition of growth in plain broth* 24 hr: Mols per 100 cc x10⁶	Mg per 100 cc	Inhibition of growth in plain broth* 48 hr: Mols per 100 cc x10⁶	Mg per 100 cc	Inhibition of growth in glucose broth† 24 hr: Mols per 100 cc x10⁶	Mg per 100 cc	Inhibition of growth in glucose broth† 48 hr: Mols per 100 cc x10⁶	Mg per 100 cc
2-Methyl-1,4-naphthoquinone	2.9	0.50	2.9	0.50	4.35	0.75	4.35	0.75
2,6-Dimethoxybenzoquinone	2.9	0.49	2.9	0.49	5.70	0.98	7.28	1.22
1,4-Naphthoquinone	5.8	0.92	5.8	0.92	10.15	1.60	13.06	2.06
1,2-Naphthoquinone	4.35	0.69	5.8	0.92				
2-Methyl-1,4-naphthol-4-amino hydrochloride	2.9	0.61	2.9	0.61	4.35	0.91	5.80	1.21
Tolu-hydroquinone					7.25	0.90	17.4	2.16
Penicillin	3‡		4‡					

* Size of inoculum—1,920 organisms.
† Size of inoculum—6,410 organisms.
‡ Florey units per 100 cc; one unit of material used equivalent to 0.0091 mg.

approximately 13 to 18 times as effective, on a weight basis, as 2-methyl-1,4-naphthoquinone or 2,6-dimethoxy benzoquinone. However, the sample of penicillin was probably not a pure substance and its molecular weight is undoubtedly higher than either of the two quinones mentioned above. Therefore, the actual effectiveness of penicillin, both on a weight basis and on a molecular basis, in relation to the antibacterial activity of the two most potent quinones is undoubtedly higher than these calculations serve to indicate. The higher amounts of the quinones required for bacterial inhibition in glucose broth in comparison to the quantities needed for the same purpose in plain broth is probably due to the fact that glucose broth is a more favorable medium for the growth of staphylococci.

There is some suggestion in Table I that the minimum antibacterial concentrations of quinones is conditioned in part by the number of organisms in the inoculum. Some additional information as to the magnitude of this factor was obtained in regard to 2-methyl-1,4-naphthoquinone using inocula of 1,500, 11,000 and 147,000 staphylococci. The minimum concentrations of this quinone required to prevent detectable growth of the organisms for 24 hours in broth were respectively 0.50, 1.0 and 1.25 mg per 100 cc.

On account of the growth characteristics

TABLE III.
In vitro Antibacterial Effects Against a Strain of Streptococcus of Quinones Compared on a Molecular and on a Weight Basis.

Compound	Minimum concentrations required for complete: Inhibition of growth: Mols per 100 cc x10⁶	Mg per 100 cc	Sterility: Mols per 100 cc x10⁶	Mg per 100 cc
2-Methyl-1,4-naphthoquinone	1.16	0.200	1.16	0.200
2,6-Dimethoxy-benzoquinone	<0.290	<0.049	<0.29	<0.049
1,4-Naphthoquinone	0.87	0.137	1.15	0.183
Tolu-hydroquinone	0.58	0.072	0.58	0.072
2-Methyl-1-naphthol-4-amino hydrochloride*	1.45	0.303	>5.8	>1.21
2-Methyl-1,4-naphthoquinone sodium bisulfite addition compound†	1.45	0.400	>5.8	>1.60

* "Synkamin" of Parke Davis.
† Abbott's "Hykinone."

of the streptococcus C203, it was necessary to use 8-hour cultures, to terminate the incubation after 16 hours, to employ 0.5% glucose broth, and to use inocula of higher counts. The experiments were otherwise carried out essentially as described above. Sterility tests were done after the incubation period, by inoculating 1 cc of the solutions into blood agar pour plates.

The results in Table III, pertaining to the first 4 substances, were obtained in the course of the experiment using inocula of 27,000 streptococci. Inocula of 153,000 organisms were used in the experiment which gave the data with respect to "Synkamin" and "Hykinone." It will be noted that several of these substances appear to exert a considerably greater antibacterial action against the C203 streptococcus than was observed in the case of the staphylococcus. None of the substances named in Table III in a concentration as high as 29.0×10^{-6} mols per 100 cc exerted any bacteriostatic or bactericidal effect against a strain of *Escherichia coli.*

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Production of Hypoprothrombinemia and Hypocoagulability of the Blood with Salicylates.*

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It has recently been reported by Link, Overman, Sullivan, Huebner and Scheel[1] that salicylic acid and sodium salicylate administered repeatedly, or in single doses, orally or intravenously, are capable of producing hypoprothrombinemia in rats. It had already been established[2,3] that salicylic acid is a degradation product of Dicumarol (3,3′ methylenebis (4-hydroxycoumarin)), which is now recognized to be a hypoprothrombinemic agent. In August of 1942 the observations of Link *et al.*[1] were made known to us, and shortly thereafter clinical investigations were begun in an attempt to reproduce the animal experiments. At the same time Dr. Shepard Shapiro of New York City Welfare Hospital, New York University, Division III, began similar studies independently.

The experimental group consisted of 31 adults, males and females, about a fourth of whom were normal individuals—nurses, technicians, and medical students—and the rest patients in the State of Wisconsin General Hospital who were afflicted with a wide variety of illnesses, arthritis of various types being the most common. These subjects received salicylates, in the form of acetylsalicylic acid or sodium salicylate in daily doses ranging from 20 to 80 grains (1.3 g to 5.3 g) for periods of 3 to 11 days.

Methods. The prothrombin time was determined on undiluted plasma with Quick's method as modified by Pohle and Stewart.[4] On each day that tests were made, one or more untreated patients was tested to serve as a control upon the technic used and the potency of the thromboplastin. Ordinarily the thromboplastin was of such potency as to result in a normal prothrombin time of 10 to 11 seconds for this group of experiments. The approximate percentages of prothrombin in human plasma as derived from the chart of Pohle and Stewart are as follows: A time of 12.5 seconds indicates a concentration of

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