a statistically significant favorable result. However, a substance to counteract an acutely lethal dose of a substance must act rapidly as well as be potent. On the contrary, Horne and Scarborough found ascorbic acid to be ineffective and hesperidin to be effective in the treatment of a patient with arsenical purpura.[1] Gorrie[8] has also reported hesperidin to be effective in the treatment of arsenical purpura.

Although we were not interested in devising a method for the assay of vitamin P, it is possible that our observations may be sufficiently consistent to be utilized for the development of a method for the assay of vitamin P active substances.

These results would indicate that it would be advisable to provide patients with an abundant source of vitamin P and C for several days prior to and during treatment with arsenicals.

Conclusions. Ascorbic acid and hesperidin methyl chalcone when used as a combined therapy afforded a definite protection in mice against the toxic effect of a single dose of intravenously administered dichlorophenarsine hydrochloride.

[8] Gorrie, D. R., *Lancet*, 1940, **1**, 1005.

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Antibacterial Properties of 4-Amino-2-Methyl-1-Naphthol Hydrochloride.*

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As previously reported[1] pyridoxamine acquires antibacterial potency following irradiation at suitable wavelength and pH. Lack of activity in irradiated and non-irradiated solutions of pyridoxine, dimer and trimer of pyridoxine,† pyridoxal and a number of pyridine and benzene derivatives demonstrated that the presence of an amino and one or two hydroxyl substituent groups is an essential prerequisite for the development of antibacterial activity. It was tentatively assumed that the antibacterial factor may be an intermediate product of oxidation.‡ Accordingly, it seemed to be of particular interest to determine the antibacterial potency of 4-amino-2-methyl-1-naphthol hydrochloride (i.e. water soluble derivative of vitamin K, Synkamin of Parke, Davis) in view of the presence of an amino substituent group and the readiness with which the compound undergoes oxidation. The results of the studies are summarized below:

Solutions of Synkamin having the highest antibacterial potency were prepared, as follows:

Tenth normal HCl solution was autoclaved and cooled to 37°C prior to use. Pure dry Synkamin powder§ and sodium bisulfite were dissolved in concentrations of 3 and 0.5 mg per ml, respectively. Immediately after preparation the solution was sealed with vaseline and stored in the refrigerator.

As may be seen from Table I, Synkamin solution prepared as above proved strongly bacteriostatic against gram positive organisms in broth and gram negative bacilli in Gladstone synthetic medium. Among the few strains tested, staphylococcus, strain H and pneumococcus, type I were highly susceptible, while *Staphylococcus hemolyticus* was refractory. As may be also noted from the same

* This investigation has been supported by grants from the Commonwealth Fund and The National Vitamin Foundation.

† The author is thankful to Dr. John V. Scudi for a supply of this compound.

[1] Shwartzman, G., and Fisher, A., *J. Biol. Chem.*, 1947, **167**, 345.

‡ Possibly due to the formation of a lactam ring between the amino and hydroxyl groups following the elimination of a molecule of water.

§ Generously supplied by Dr. L. A. Sweet of Parke, Davis and Company.

TABLE I.
Effect of Synkamin in N/10 HCl Containing 0.05% $NaHSO_3$, Sealed with Vaseline.

Organism tested	Medium	Minimal amount of Synkamin: Complete inhibition	Minimal amount of Synkamin: 25 to 50% inhibition
Staphylococcus, Strain H	Broth	1.5-2 γ	1 γ
Streptococcus hemolyticus, Strain 870	"	—	—
Pneumococcus, Type I	"	2.5 γ	
E. coli, Strain 42	Gladstone synthetic medium	3 γ	2 γ
" " " L	" " "	2.5 γ	1 γ
S. binns	" " "	1.5 γ	0.8 γ
S. paratyphi B.	" " "	2.5 γ	1.5 γ
B. friedlanderi, Type A	" " "	35 γ	
E. coli, Strain 42	Broth	—	—
S. binns	"	—	—
Meningococcus, Group I	Synthetic medium[2]	0.75 γ	
" " "	Broth	2.5-3 γ	

— = No inhibition with 100 γ of Synkamin.
The composition of Gladstone synthetic medium and methods of determination of bacteriostatic activity and the size of inocula were the same as previously described.[1]

table and additional experiments not recorded in the table, gram negative bacilli (with the exception of *B. friedlanderi*, type A.) were markedly susceptible to Synkamin. All the gram negative bacilli tested were resistant to the compound in broth, suggesting the presence of an antagonistic factor in this medium. The antagonistic effect of broth was less pronounced with meningococcus, type I, the organism being approximately only 4 times more susceptible in synthetic medium than in broth. In addition to broth, casein hydrolysate and blood serum proved antagonistic to the activity of Synkamin against *E. coli*. Various concentrations of the substances were added to Gladstone synthetic medium containing different amounts of Synkamin. The mixtures were inoculated with *E. coli*, strain 42. There was obtained 50% reversal of the antibacterial activity in the following ratios of dry weights, namely, one part of Synkamin to 125 parts of casein hydrolysate, 65 parts of mouse serum and 50 parts of rabbit serum. It remains as yet unknown whether staphylococcus would show greater susceptibility in synthetic medium than in broth. No reversal of antibacterial activity was noted following the addition of casein hydrolysate and serum to broth cultures of staphylococcus, strain H. The nature of the factor antagonizing inhibition of *E. coli* was not yet identified.

The experiments about to be described were done in order to determine the effect of oxidation by air upon the antibacterial activity of Synkamin. The preparations were tested against *E. coli* in Gladstone synthetic medium and staphylococcus, strain H in broth. Solutions of Synkamin in N/10 HCl, in concentration of 3 mg per ml with and without vaseline seal were stored in the dark at 4°C. Twenty-four to 48 hours following preparation there occurred a 25% loss of activity in the non-sealed preparation. The titer remained stationary during the following 6 days. There was almost complete loss of activity 10 days following preparation. In the vaseline sealed preparations the titer was unchanged for the entire period of observation, i.e. 3 to 4 weeks.

Immediately upon preparation solutions containing 3 mg of Synkamin and 1 mg of sodium bisulfite in N/10 HCl gave complete inhibition of *E. coli* in concentration of 2-3 γ of Synkamin per ml of synthetic medium. On exposure to air for 4 days at 4°C there occurred a 25% loss of activity. On the other hand, addition of 2 mg of sodium bisulfite to 3 mg of Synkamin produced an immediate 40% decrease of activity which was completely restored on air exposure at 4°C for 4 days. The effect of the reducing substance upon the activity of Synkamin against staphylococcus, strain H was not as clear-cut, possibly in view of the interference of broth with the reduction.

[2] Frantz, I. D., *J. Bact.*, 1942, **43**, 757.

Additional experiments are under progress in order to investigate this relationship.

Additions of cystine, 1 mg, methionine, 3 mg. and methionine sulfoxide,‖ 5 mg, to 3 mg of Synkamin in N/10 HCl, per ml failed to antagonize the effect of Synkamin upon *E. coli,* strain 42 and staphylococcus, strain H.

It is obvious from the above experiments that the antibacterial activity of Synkamin described is intimately related to air oxidation. Excessive oxidation brings about loss of activity. Reduction with sodium bisulfite results in reversible decrease in potency which is restored on exposure to air. It is suggestive that the antibacterial activity of Synkamin is due to an intermediate state of oxidation by air which possibly takes place immediately upon dissolving the substance. The antibacterial potency can be maintained at a constant level by timely exclusion of air from an oxidized solution or by additional oxidation of a reduced solution.

The effect of Synkamin was compared with that of 2 other water soluble derivatives of vitamin K, sodium-2-methyl-1,4-naphthoquinone diphosphate (Synkayvite, Hoffmann-La Roche) and 2-methyl-1,4-naphtho-hydroquinone-3-sodium-sulfonate (Hykinone, Abbott).¶ The solutions were made similarly to Synkamin, with and without vaseline seal, and tested against staphylococcus, strain H in broth and *E. coli,* strain 42 in Gladstone synthetic medium. All solutions of Synkayvite were totally devoid of antibacterial activity against these organisms in concentrations 1-200 γ per ml. Immediately following preparation and following storage at 4°C, Hykinone showed no effect upon *E. coli.* After one week storage at room temperature the compound inhibited *E. coli* in the concentration of 40 γ per ml of synthetic medium. Staphylococcus, strain H, was completely inhibited by 10 γ and partially inhibited by 5 γ per ml of broth. Thus, Synkayvite possessed no activity under conditions described, while the potency of Hykinone was distinctly lower than that of Synkamin. The antibacterial effect also seems to be significantly greater with Synkamin than with vitamin K derivatives studied by previous authors,** i.e. 2-methyl-1,4-naphthoquinone and derivatives containing chloro, methyl and methoxy substituent groups.

Summary. The points of interest of the studies embodied in this note are as follows: 4-amino-2-methyl-1-naphthol hydrochloride possesses marked antibacterial activity against gram positive and gram negative organisms.

The compound is strongly effective against gram positive organisms in presence of broth, casein hydrolysate and blood serum, and against gram negative organisms in synthetic medium. The activity against the latter organisms is antagonized in broth medium. Casein hydrolysate and mouse and rabbit sera antagonize the antibacterial activity of Synkamin against *E. coli* in a significantly lesser degree than broth, namely, in dry weight ratios of 125:1, 65:1, 50:1, respectively.

The presence of an amino substituent group and partial oxidation of the substance under carefully controlled conditions play an important role in the antibacterial activity described.

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‖ Kindly made available by Dr. Joseph Seifter of Wyeth Institute.

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** No detailed comparison can be presented in this brief communication. Reports of previous investigations are listed under References.[3]

3 Fosdick, L. S., Fancher, O. E., and Calandra, I., *Science,* 1942, **96**, 45; Armstrong, W. D., Spink, W. W., and Kahnke, I., PROC. SOC. EXP. BIOL. AND MED., 1943, **53**, 230; Wooley, D. W., PROC. SOC. EXP. BIOL. AND MED., 1945, **60**, 225; Colwell, A., and McCall, M., *J. Bact.,* 1946, **51**, 659; and Hoffmann-Ostenhof, O., *Science,* 1947, **107**, 549.