ness and insomnia were less frequent in the control group. All side reactions mentioned occurred more frequently when the dose of pyribenzamine was doubled.

This study was prepared at Walter Reed General Hospital while the author was in the Army Medical Corps.

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Protective Action of Vitamins C and P Against Dichlorophenarsine Hydrochloride (Clorarsen).

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This study was undertaken specifically to ascertain whether the use of vitamins C and P in combination would protect against the acutely toxic effect of intravenously injected dichlorophenarsine hydrochloride in mice. It was thought that if these vitamins exerted a favorable effect on capillary fragility it might be demonstrated by the manifestation of a decrease in the acute toxicity of arsenic, since arsenic is a potent capillary poison.

The literature dealing with decreased capillary resistance as one of the toxic manifestations of antisyphilitic therapy has been reviewed by Horne and Scarborough.[1] The apparently protective action of vitamin C against the toxicity of arsenicals has been commented on by several groups of authors.[2,3,4] Scarborough and Stewart have reported that vitamin P (hesperidin) treatment increased capillary resistance in erythema and dermatitis due to arsenic and bismuth.[5]

Goldforb[6] suggested that the vitamin in the form of aqueous extracts of whole lemon might prevent arsenical encephalopathy in patients receiving intensive arsenical therapy. And, Goldstein, Stalman and Goldforb[7] found that treatment with the methyl chalcone of hesperidin decreased the mortality of a standard dose of mepharsen in rabbits from 90 to 57%, a difference that was of questionable significance ($X = 2.9$).

In a preliminary study on groups of control and treated mice (Table I), it was found that vitamin C alone (2.2 millimoles per millimole of dichlorophenophenarsine hydrochloride) had a favorable, but not statistically significant effect. The same was true when similar groups of mice were treated with hesperidin methyl chalcone alone (1 mg each daily for 9 days previous and 3 days following the injection of the arsenical).

For these reasons it was decided to investigate the protective effect of a combination of these two substances on the acute toxicity of a standard dose of intravenously administered dichlorophenarsine hydrochloride.

Method. Adult female white mice, weighing from 17 to 24 g (average weight, 21 g apiece in each group) maintained on a diet of Purina Checkers plus lettuce were used. Injections of dichlorophenarsine hydrochloride (clorarsen, Squibb) dissolved in saline were made into the tail vein, and all animals were observed for 3 days after injection, since those not

[1] Horne, G., and Scarborough, H., *Lancet*, 1940, **2**, 66.

[2] Sulzberger, M. B., and Oser, L. B., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 716.

[3] Bundesen, H. N., Aron, H. C. S., Greenbaum, R. S., Farmer, C. J., and Abt, A. F., *J. Am. Med. Assn.*, 1941, **117**, 1692.

[4] McChesney, E. W., Barlow, O. W., and Klinck, G. H., *J. Pharm. and Exp. Therap.*, 1944, **80**, 81.

[5] Scarborough, H., and Stewart, C. P., *Lancet*, 1938, **2**, 610.

[6] Goldforb, A. E., *Arch. Dermat. and Syph.*, 1941, **43**, 536.

[7] Goldstein, D. H., Stalman, A., and Goldforb, A. E., *Science*, 1943, **98**, 245.

TABLE I.

Results Showing the Protective Action of Vitamins P and C Given in Combination Against the Toxicity of Dichlorophenarsine Hydrochloride.

Group No.	Control groups				Lot of hesperidine methyl chalcone	Group treated with a combination of Vitamins C and P.				Statistical significance
	No. of mice	No. dead	No. survived	% survival		No. of mice	No. dead	No. survived	% survival	
I	40	36	4	10	No. 1369	40	7	33	82.5	$X^2 = 39.1$
II	20	17	3	15	" 1369	15	3	12	80.0	$X^2 = 14.7$
Subtotal Lot No. 1369	60	53	7	11.6		55	10	45	81.8	$X^2 = 57.0$
III	16	9	7	44	" 1653	17	0	17	100	$X^2 = 11.9$
IV	20	11	9	45	" 1653	21	8	13	62	$X^2 = 1.17$
V	15	8	7	46.7	" 1653	19	1	18	94.7	$X^2 = 12.7$
Subtotal Lot No. 1653	51	28	23	45.1		57	9	48	84.2	$X^2 = 18.2$
	—	—	—	—		—	—	—	—	
Total	111	81	30	27.0		112	19	93	82.0	$X^2 = 70.6$
	Treated with Vitamin C alone.					Treated with hesperidine methyl chalcone alone				
IA	40	29	11	27.5	No. 1369	40	23	17	42.5	

killed by the arsenical in that length of time were eating well.

In the treated animals, vitamin P was administered as purified methyl chalcone of hesperidin. This substance was kindly made available by Dr. W. Biehm, Medical Director of Abbott Laboratories. When uncertainty existed regarding whether all the arsenical was actually gotten into the vein, the mouse in question was discarded from the series.

The Control Groups. All mice whether in the control or treated groups received a dose of 27 mg of dichlorophenarsine hydrochloride per kilo of body weight. This dose was used because preliminary experiments indicated that it approximated an L. D. 85 dose of the arsenical when injected into the tail vein.

The Treated Groups. These groups received a daily subcutaneous dose of 1 mg apiece of methyl chalcone of hesperidin in saline each day for 9 days previous to and 3 days following the injection of the above dosage of dichlorophenarsine hydrochloride which also contained 2.2 millimoles of ascorbic acid per millimole of dichlorophenarsine hydrochloride. The dosage of ascorbate was chosen for convenience and because the observations of McChesney, Barlow and Klinck[4] have indicated that it confers protection. The dose of vitamin P represented an average dose of 47.6 mg of vitamin per kg of body weight per day and was chosen arbitrarily. It is 36% greater than the dose used by Goldstein and his associates.[7]

Results. The results on the different control and treated groups are shown in Table I. It is to be noted that in each of the 5 groups of tests the treatment provided a significant protection except in the case of Group 4. It should also be noted that there is a difference in the results obtained in Groups I and II, as compared with those in Groups III, IV, and V. This may be attributed to the fact that different lots of both the vitamin and arsenical were used. Unquestionably the second lot of the arsenical was more toxic than the first lot.

Discussion. It is possible that if more mice had been used ascorbic acid alone or hesperidin methyl chalcone alone would have yielded

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.