period of 50 min. was required to destroy all of the organisms on glass slides whereas corresponding cultures on cotton were completely destroyed after an exposure of only 25 minutes.

Conclusions. It may be stated that the increased efficiency of formaldehyde as a germicide was not due to any effect of the vacuum on the gas but rather to the ability of the vacuum to increase the penetration of the gas. Formaldehyde is chiefly a surface disinfectant. The increased penetration in the presence of a vacuum made it possible for the gas to reach the organisms. The vacuum principle is capable of producing a very deep penetration of the gas into porous materials. The method is very efficient, cheap, easily performed, and indispensable for the sterilization of objects likely to be injured by the application of dry or moist heat.

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Reactions of 2-Methyl-1, 4-Naphthoquinone (Menadione) with Whole Blood and Plasma *in vitro*.

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Recently Seligman and coworkers[1] reported that vitamin K_1 has such a persistent and prolonged action that a single small dose is adequate for the treatment of even severe cases of hypoprothrombinemia, and may well do the work of repeated doses of other agents. This point has been emphasized by Fieser.[2] In the course of our work on the development of an oxidation-reduction method for the determination of vitamin K_1[3] it was found that the vitamin is remarkably stable in whole blood, but menadione is rapidly destroyed, and further menadione, unlike the vitamin, causes a marked methemoglobin formation.

Experimental. An oxalated sample of freshly drawn dog blood was divided into three 30 cc samples. 1.5 mg of 2-methyl-1,4-naphthoquinone, an equivalent weight of 2,3-dimethyl-1,4-naphtho-

1 Seligman, A. M., Hurwitz, A., Frank, H. A., and Davis, W. A., *Surg. Gynecol. Obstet.*, 1941, **78**, 686.

2 Fieser, L. F., *Ann. Int. Med.*, 1941, **15**, 648.

3 Scudi, J. V., and Buhs, R. P., *J. Biol. Chem.*, 1941, **141**, 451. (See also paper in press.)

quinone, or an equivalent weight of vitamin K_1, each dissolved in 0.5 cc of ether was added to the blood samples. As with other quinones,[4] when the samples were mixed, the blood containing the menadione rapidly darkened in color, and after 15 to 20 minutes 50% of the blood pigment was found, spectroscopically, to be present as methemoglobin. Under these conditions neither vitamin K_1 nor 2,3-dimethyl-1,4-naphthoquinone caused a methemoglobincythemia, possibly because of cell impermeability.[5]

Twenty minutes after the preparation of the blood samples, 5 cc aliquots were added dropwise to 70 cc of boiling absolute alcohol. The mixtures were cooled; 40 cc of absolute ether was added, and the mixtures were shaken in the absence of light. Volumes were adjusted to 150 cc with alcohol and the samples were filtered. Aliquots of the filtrates were concentrated under nitrogen *in vacuo* and butanol was added to transfer the quinones to butanol without evaporation to dryness. The butanol solutions were then analyzed as previously described.[3] Pure solutions of menadione gave 100% recoveries. In the experiments with blood, quantitative recoveries of the added vitamin K_1, and the 2,3-dimethyl-1,4-naphthoquinone were obtained. The analytical method is capable of detecting 1 γ per cc of solution, and the concentrates analyzed should have contained 10 to 20 γ per cc, but no menadione was found. Nor were recoveries effected following peptic hydrolysis of the blood proteins even though a variety of extraction procedures were tried.

Similar experiments performed with fresh oxalated plasma gave 40 to 50% recoveries of added menadione, while 1- and 2-day-old samples of plasma gave recoveries of 60 to 70%. The menadione caused the plasma samples to acquire a deep yellow color upon standing in the absence of light.

Summary. When added to whole, unlaked blood, menadione causes a marked methemoglobin formation, and the drug is rapidly converted to some other substance (or substances). Assays with vitamin K-deficient chicks showed that these reactions cause a marked loss in the antihemorrhagic activity of the menadione. Vitamin K_1, unlike menadione, does not produce methemoglobin under comparable conditions, and the vitamin is remarkably stable in whole blood.

[4] Austin, J. H., and Drabkin, D. L., *J. Biol. Chem.*, 1935, **112**, 67.

[5] Van Slyke, D. D., and Vollmund, E., *J. Biol. Chem.*, 1925, **66**, 415.