sized by the isolation of such a material with a definite, though slightly diffuse boundary from crude vaccines less than 48 hours old (infectivity diminished at least 5 decimal dilutions). Older vaccines fractionated at pH below 7.0, however, yield materials that show only suggestions of a boundary or completely diffuse shadows in the sedimentation pattern where boundaries corresponding to this approximate particle size should appear.

## 11293

## Water-Soluble Antihemorrhagic Compounds.

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The interest in vitamin K analogues suitable for parenteral medication is evident from the current literature. Usually patients with hypoprothrombinemia due to obstructive jaundice receive orally vitamin K and bile or bile salts. Frequently these latter substances are badly tolerated and it seems that they are not needed, if the antihemorrhagic material is administered parenterally. In cases of severe hepatic insufficiency vitamin K orally is apparently not beneficial, but intravenous injections of a vitamin K analogue, such as e.g. 2-methyl-1,4-naphthoquinone, may be effective in raising the prothrombin content of the blood, as reported by Rhoads and Fliegelman.[1]

The first synthetic antihemorrhagic substance to be employed parenterally in the clinic was phthiocol.[2,3] Relatively large doses (50-100 mg) are required, because the potency of this compound is small.[4] Flynn and Warner[5] determined that phthiocol had but 1/500 the activity of methylnaphthoquinone, and we had reported pre-

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1 Rhoads, J. E., and Fliegelman, M. T., *J. Am. Med. Assn.*, 1940, **114**, 400.

2 Smith, H. P., Ziffren, S. E., Owen, C. A., and Hoffman, G. R., *J. Am. Med. Assn.*, 1939, **113**, 380.

3 Butt, H. R., Snell, A. M., and Osterberg, A. E., *Proc. Staff Meeting Mayo Clin.*, 1939, **14**, 497.

4 Ansbacher, S., and Fernholz, E., *J. Am. Chem. Soc.*, 1939, **61**, 1924.

5 Flynn, J. E., and Warner, E. D., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 190.

viously[6] an activity ratio of 1:1000 and even of 1:4000, when an especially purified sample of synthetic phthiocol was used.

As already mentioned, methylnaphthoquinone in an aqueous medium is being employed clinically for intravenous medication. Aqueous or physiologic salt solutions containing 1 mg in 10 ml appear to be stable when kept in a refrigerator, and methylnaphthohydroquinone is even more soluble (3 to 4 times) in water. Recently Dann[7] found that oil solutions are roughly 3 times as active as aqueous ones. Our assays (Table I) show that methylnaphthoquinone, when administered orally, is about twice as potent in water as in oil, and confirm our previous observation[8] that the oil, used as a medium for administering vitamin K, interferes to a noticeable degree with the potency. Intravenously the aqueous solution is about as active as the oil solution orally.

The sodium salt of 2-methyl-1,4-naphthohydroquinone disulfuric acid was synthesized by Fieser and Fry[9] who stated that it showed antihemorrhagic activity at a level of 2γ. We found (Table I) that this compound has a potency of 1 unit in about 25γ, and 5γ is fully effective in an 18-hour test (Table II).

Sodium 2-methyl-1,4-naphthohydroquinone diphosphate was prepared by Fieser and Fry[9] and by Foster, *et al.*[10] The latter authors stated that it had an oral effectiveness lying below 2γ. Our data (Table I) show that this substance has a potency of 1 unit in about 10γ, and 5γ is the minimum fully effective dose when the test period is prolonged to 18 hours (Table II). Compared with methylnaphthoquinone, this compound is but 1/20 as active. The results presented in Tables I and II do not agree with those of Foster, *et al.*,[10] who expressed the opinion that this sodium salt represents probably the most active antihemorrhagic substance known when compared on a molecular basis with methylnaphthoquinone. Furthermore, our observations do not support the speculation of these investigators as to whether the antihemorrhagic effects of 2-methyl-1,4-naphthoquinone, of its reduced form, and of like substances, are not mediated through a phosphoric ester.

We wish to report on still another water-soluble antihemorrhagic compound, 2-methyl-1,4-naphthylene-dioxy diacetic acid. It has a potency of 1 unit in about 2 mg (Table I), and 100γ is effective in

[6] Fernholz, E., and Ansbacher, S., *Science*, 1939, **90**, 315.

[7] Dann, F. P., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 663.

[8] Ansbacher, S., *J. Nutrition*, 1939, **17**, 303.

[9] Fieser, L. F., and Fry, E. M., *J. Am. Chem. Soc.*, 1940, **62**, 228.

[10] Foster, R. H. K., Lee, J., and Solmssen, U. V., *J. Am. Chem. Soc.*, 1940, **62**, 453.

TABLE I.
Vitamin K Assays.

| Substance | Mode of administration | Vehicle | Response* > 1 unit† γ | = 1 unit γ | < 1 unit γ |
|---|---|---|---|---|---|
| 2-methyl-1,4-naphthoquinone‡ | orally | oil | 3/4 | 1/2 | 1/4 |
| | " | water | 1/2 | 1/4 | 1/10 |
| | intraven. | " § | 3/4 | 1/2 | 1/4 |
| Sodium 2-methyl-1,4-naphthohydroquinone diphosphate | orally | " | 12.5 | 10 | 7.5 |
| Sodium 2-methyl-1,4-naphthohydroquinone disulfate | " | " | 30 | 25 | 20 |
| Phthiocol | " | capsules | 600 | 500 | 400 |
| Purified phthiocol | " | " | 2250 | 2000 | 1750 |
| 2-methyl-1,4-naphthylene-dioxy diacetic acid | " | water | 2250 | 2000 | 1750 |

*A minimum of twenty severely vitamin K-deficient chicks was used for each dose level.
†Ansbacher unit (*J. Nutrition*, 1939, **17**, 303).
‡Methylnaphthoquinone or methylnaphthohydroquinone.
§Water or physiologic salt solution.

TABLE II.
Minimum Effective Doses in 6- and 18-hour Tests.

| Substance | 6 hr γ | 18 hr γ | Ratio |
|---|---|---|---|
| 2-methyl-1,4-naphthoquinone* | 1/2† | 1/4† | 2:1 |
| | 1/4‡ | 1/8‡ | |
| Sodium 2-methyl-1,4-naphthohydroquinone diphosphate | 10 | 5 | 2:1 |
| Phthiocol (purified) 2-methyl-3-hydroxy-1,4-naphthoquinone | 2000 | 1000 | 2:1 |
| Sodium 2-methyl-1,4-naphthohydroquinone disulfate | 25 | 5 | 5:1 |
| Vitamin $K_1$, 2-methyl-3-phytyl-1,4-naphthoquinone | 15 | 1 | 15:1 |
| 2-methyl-1,4-naphthylene-dioxy diacetic acid | 2000 | 100 | 20:1 |

*Methylnaphthoquinone or methylnaphthohydroquinone.
†Orally in oil or intravenously in an aqueous medium.
‡Orally in water.

an 18-hour test (Table II). This substance was prepared in the following manner:

To 15 g of 2-methyl-1,4-naphthohydroquinone and 16.3 g of monochloracetic acid, 13.8 g of sodium hydroxide in 350 ml of water was added, the mixture refluxed for 6 hours under nitrogen and allowed

to stand overnight. The product was then diluted with an equal volume of water and 10% hydrochloric acid added until an oil separated which soon solidified. It was removed by filtration, taken up in a 10% sodium carbonate solution, warmed in the presence of a decolorizing charcoal, filtered and reprecipitated with dilute hydrochloric acid. A pinkish powder with a m.p. of 204-10° was thus obtained. It was dissolved in a 10% sodium bicarbonate solution, treated three times with decolorizing charcoal, and reprecipitated from the almost colorless solution with dilute hydrochloric acid. The final product was a white powder, m.p. 217-218°; yield 7.5 g (30%).

*Analysis.* Calculated for $C_{15}H_{14}O_{16}$: C, 62.04%; H, 4.86%.
Found: C, 61.38%; H, 4.92%.

Other water-soluble vitamin K substitutes have been prepared and studied clinically. According to Butt *et al.*,[11] the 1,4-dihydroxy-2-methyl-3-naphthaldehyde of Binkley and coworkers[12] is effective at a level of 10 mg, and Broun[13] found that the 2-methyl-4-amino-1-naphthol hydrochloride of Doisy and associates[14] has a definite effect at 5 mg. Synthetic vitamin $K_1$ is practically insoluble in water. It has been used by Frank, *et al.*[15] in form of a colloidal suspension in glucose and was active at 10 mg. These data would seem to confirm the statement by Rhoads and Fliegelman[1] that "2-methyl-1,4-naphthoquinone appears to be the most potent agent for the treatment of prothrombin deficiency so far employed clinically."

*Summary.* Methylnaphthoquinone or methylnaphthohydroquinone is the most active vitamin K compound known. It is as effective intravenously in an aqueous medium as orally in oil solution. *Per os* its potency is greater in water than in oil. The phosphate derivative is considerably less active. The sulfate is not as potent and not as rapidly absorbed by the organism as the phosphate. A new water-soluble vitamin K-active compound is described.

11 Butt, H. R., Snell, A. M., Osterberg, A. E., and Bollman, J. L., *Proc. Staff Meeting Mayo Clin.*, 1940, **15**, 69.

12 Binkley, S. B., Cheney, L. C., Holcomb, W. F., MacCorquodale, D. W., Thayer, S. A., and Doisy, E. A., 98th Meeting, Am. Chem. Soc., Boston, Sept. 12, 1939.

13 Broun, G. O., 12th Ann. Meeting, Central Soc. Clin. Res., Chicago, Nov. 3 and 4, 1939 (*J. Am. Med. Assn.*, 1940, **114**, 440).

14 Doisy, E. A., MacCorquodale, D. W., Thayer, S. A., Binkley, S. B., and McKee, R. W., Nation. Acad. Sci., Brown U. Meeting, Oct. 23-25, 1939 (*Science*, 1939, **90**, 407).

15 Frank, H. A., Hurwitz, A., and Seligman, A. M., *New England J. Med.*, 1939, **221**, 975.