VITAMIN K ACTIVITY OF SYNTHETIC PHTHIOCOL

ALMQUIST and Klose[1] recently announced that pure synthetic phthiocol (2-methyl-3-hydroxy-1,4-naphthoquinone) has antihemorrhagic activity. With a sample of phthiocol, synthesized according to Anderson and Newman,[2] we were able to confirm the fact that phthiocol has some vitamin K activity.[3] Thayer *et al.*[4] have also published a confirmatory report. It is now fairly well established that a variety of naphthoquinones cure the vitamin K deficiency of chicks in a single dose of an order of magnitude of a milligram. The sample of phthiocol reported in our first communication had an activity of one unit[5] in 0.5 mg. We have since found that the vitamin K activity of phthiocol prepared according to Anderson and Newman is to a large extent due to traces of an impurity, presumably 2-methyl-1,4-naphthoquinone, which can be removed by washing its solution in alkali with ether. A sample so purified was found to have one unit in 2 mg, and is therefore only one fourth as active as the original sample. The melting point is not changed by this special purification procedure.

This is understandable in view of the unusually high potency of 2-methyl-1,4-naphthoquinone and the diacetate of the corresponding hydroquinone which serve as a starting material for the preparation of phthiocol. Since our original publication,[6] we have investigated the potency of these two compounds more extensively. The minimum effective dose is even lower than that of vitamin K_1.[7] While 2 γ of vitamin K_1 are required for a unit as used in our laboratory, the diacetate of 2-methyl-1,4-naphthohydroquinone requires 1 γ and 2-methyl-1,4-naphthoquinone only 0.5 γ. The duration of the curative effect of a single dose of 0.5 γ of the methyl-naphthoquinone dissolved in 0.1 cc of cod liver oil given to severely deficient chicks with a blood clotting time of over 90 minutes is illustrated by Table 1.

TABLE 1
DURATION OF THE CURATIVE EFFECT OF 0.5 γ OF 2-METHYL-1,4-NAPHTHOQUINONE

Chick No.	Weight gr.	Clotting time (minutes) after 6	18	48	72	96 hours
7316	60	2	1	2	5	7
7350	75	5	2	2	6	6
7366	70	3	1	2	>30	>30
7393	60	3	<½	2	4	8
7403	65	3	1	2	3	7
7414	70	6	2	2	5	>30
7430	65	3	1	2	8	>30
7449	70	2	2	2	5	>30

The activity of 2-methyl-1,4-naphthoquinone is so high that a contamination of the repurified phthiocol with 0.025 per cent. of the substance would account for the vitamin K activity. We have, therefore, submitted our phthiocol to chromatographic adsorption, using a benzene solution and calcium sulfate for adsorbent. The substance was readily adsorbed and drawn out to a homogeneous orange column on continued washing. The column was then divided into two equal parts and both parts separately eluted with ether. The activity of both fractions was equal and the same as before adsorption, showing that no fractionation had occurred. This degree of activity is probably a genuine property of phthiocol, although a biological assay of natural phthiocol would still be of interest.

A report of a successful application of phthiocol to a patient with low prothrombin time has already appeared in the literature.[8] Also 2-methyl-1,4-naphthoquinone is now being studied for its effect in raising the prothrombin content of the blood of such patients. In all of a considerable number of cases it has been found effective in a daily dosage of one milligram.

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[13] In another paper (footnote 12), a positive reaction with the Schiff reagent was erroneously referred to as a positive Feulgen test. On closer examination it was found that, as in the case of the tumor fraction, the intense reaction obtained with the fuchsin-sulfurous solution was given by the lipoid components of the chick embryo material, whereas a typical reaction for thymonucleic acid was apparently absent.

[14] The work has now been extended to a variety of other avian and mammalian tissues, especially mouse embryo and mouse tumors. By the use of the same procedure it has been possible to isolate from these tissues a fraction, likewise composed of small granules, which presents physical and chemical properties similar to those already described for chicken tumors and chick embryo fractions. The material separated and purified in the centrifuge may represent as much as 2 to 8 per cent. of the whole cellular body. These observations indicate that a phospholipid-ribose nucleoprotein complex is probably a general constituent of normal and tumor cells.

[1] H. J. Almquist and A. A. Klose, *Jour. Am. Chem. Soc.*, 61: 1611, 1939.

[2] R. J. Anderson and M. S. Newman, *Jour. Biol. Chem.*, 103: 405, 1933.

[3] S. Ansbacher and E. Fernholz, *Jour. Am. Chem. Soc.*, 61: 1924, 1939.

[4] S. A. Thayer, L. C. Cheney, S. B. Binkley, D. W. MacCorquadale and E. A. Doisy, *ibid.*, 1932.

[5] S. Ansbacher, *Jour. Nutrition*, 17: 303, 1939.

[6] S. Ansbacher and E. Fernholz, *loc. cit.*

[7] S. A. Thayer, L. C. Cheney, S. B. Binkley, D. W. MacCorquadale and E. A. Doisy, *loc. cit.*

HUMAN VACCINATION AGAINST EQUINE ENCEPHALOMYELITIS VIRUS WITH FORMOLIZED CHICK EMBRYO VACCINE[1]

THE pathogenicity of equine encephalomyelitis virus for man[2] has been proved by recognition of infections

[8] H. P. Smith, S. E. Ziffren, C. A. Owen and G. R. Hoffman, *Jour. Am. Med. Assoc.*, 113: 383, 1939.

[1] This work was supported by a grant from Lederle Laboratories, Pearl River, N. Y.

[2] K. F. Meyer, *Ann. Int. Med.*, 6: 645, 1932.