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.*

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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.

with the virus under natural conditions[3] and following laboratory contact with virus-diseased chick embryo tissues.[4,5] The occurrence of unrecognized infection in man has been demonstrated,[5] and in a recent study[6] of the incidence of such subclinical infections we have examined 184 human sera,[7] 69 of which were from individuals exposed to the Eastern strain virus. In 23 of these 69 sera antibodies sufficient to neutralize 100 to 1,000 infective doses of virus were found, and in one instance the serum fixed complement in the presence of drawn prior to the first vaccination and after 7, 14 and 21 days.

Slight general reactions were observed in a few of 89 volunteers vaccinated. Sharp transient pain was experienced locally in most instances, followed occasionally by low-grade dull pain for from one to three days.

Sera from 23 of those vaccinated have been tested[9] for neutralizing antibodies against the Eastern strain virus, and a typical protocol is shown in Table 1. In

TABLE 1

MOUSE PROTECTION TESTS AGAINST EQUINE ENCEPHALOMYELITIS VIRUS (EASTERN STRAIN) WITH SERUM OF WOMAN VACCINATED WITH FORMOLIZED CHICK EMBRYO VACCINE

Case	Serum	Date bled	Date vaccinated	Amount vaccine	Number of deaths in 4 injected mice				
					Virus dilution				
					10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
L. H.	a (control)	May 2	May 2	1.0 cc	4	3	2	2	0
	b	May 9	May 9	2.0 cc	4	4	3	1	0
	c	May 16			1	1	0	0	0
	d	May 23			0	0	0	0	0

Eastern strain virus purified by ultracentrifugation. Similar though less definite results were obtained in parallel studies with the Western strain. These findings have introduced the problem of protective vaccination, especially in workers frequently exposed to virus-diseased tissues and animals. We have vaccinated a group of men and women with formolized chick embryo vaccine[8] and have studied the probable effectiveness of the procedure by the examination of their sera for the presence of neutralizing antibodies.

Injections were made with a "bivalent" vaccine, prepared in the routine way for the immunization of horses. Vaccines consisting of 40 per cent. suspensions of diseased chick embryo tissue formolized to 0.4 per cent. were prepared separately for the Eastern strain and Western strain viruses. These were centrifuged at low speed, mixed in equal parts and used without further treatment. Two injections were made with an interval of 7 days. In some instances 1.0 cc was given as the first dose and 2.0 cc for the second. The majority, however, received two doses of 2.0 cc each. Routine injections were made deep in the gluteal muscles in two areas to lessen reactions to the formalin. Injections into the deltoid muscles of three individuals caused painful local reactions. Blood was 9 of the 23, serum taken 7 days after the first injection seemed to protect mice. In every case after 14 days, antibodies nearly as effective as those after 21 days were observed. After the latter interval the antibody content was sufficiently great to neutralize completely 100 to 1,000 infectious units of virus, except in one individual, whose serum neutralized only 10 units. In one instance, the serum drawn 21 days after a single injection of 1.0 cc vaccine protected against 1,000 infectious units.

The crude bivalent vaccine was thus found to be effective in the induction of antibodies against the Eastern strain virus, and to cause little discomfort. Vaccines containing less formalin and chick tissue debris may prove as effective and more useful for human vaccination.[10]

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[3] L. D. Fothergill, J. H. Dingle, S. Farber and M. L. Connerley, *New England Jour. Med.*, 219: 411, 1938.

[4] R. R. Case, personal communication, Dr. R. W. G. Wyckoff.

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