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Kofler's Quinone and Ubiquinone Assayed for Vitamin K Activity by the Curative Blood Clotting Method

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With 1 table

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The increasing amount of information about the occurrence and biochemical function of KOFLER's quinone and ubiquinone has necessitated a renewed examination of their behavior in the biological vitamin K assay. If the vitamin K activity of these substances is not nil or negligible it might be a source of error in the determination of vitamin K in tissue or tissue elements, especially in plant tissue, where KOFLER's quinone may occur in amounts many times that of vitamin K (1).

We have therefore determined the vitamin K activity of the following compounds:

KOFLER's quinone with a side chain of 45 carbon atoms[1]). Ubiquinone with a side chain of 50 carbon atoms[2]). 2,3-dimethyl-5-phytyl-benzoquinone[3]).

All three substances were kindly furnished by F. Hoffmann – La Roche & Co., A. G., Basle.

Three different samples of KOFLER's quinone were used, viz: one prepared from maple leaves, one prepared semi-synthetically, i. e. the side chain originated from solanesol isolated from tobacco leaves, and one of unspecified origin but identified by melting point and absorbance.

The details of the method were as described by DAM, KRUSE & SØNDERGAARD (2). The method uses chicks reared on a vitamin K-free diet. The test substance is given either mixed with sucrose and formed into tablets, or in solution. A few hours before and 20 to 22 hours after the ingestion the coagulation time of blood taken from the carotid artery is determined under standard conditions. If substantial shortening of the coagulation time is obtained the results may be compared with those found with varying doses of menadione.

The results are shown in table 1.

The amounts of the quinones given are very high in view of the fact that 0.03 micrograms of menadione per gram body weight has constantly been found to produce normal coagulation time. Nevertheless a substantial re-

[1]) 2,3-dimethyl-5-solanesyl-benzoquinone. Other designations for KOFLER's quinone are: Q_{254} and Plastoquinone.

[2]) 2,3-dimethoxy-5-methyl-benzoquinone having as substituent in the 6-position a polyisoprenoid side chain analogous to the side chain in KOFLER's quinone but one isoprenoid unit longer. Other designations for this ubiquinone are: Q_{275}, $Q_{(10)}$, and Coenzyme Q.

[3]) Synthetic phytyl analogue of KOFLER's quinone.

duction of the coagulation time was obtained only in one case, namely with the largest dose of KOFLER's quinone prepared from maple leaves. A result of the same order of magnitude could have been produced by the same amount of an inactive substance contaminated with 0.002 per cent of menadione or about 0.005 per cent of vitamin K_1.

Table 1

Substance tested	Form of administration	Weight of chick g	Amount of substance mg	Amount of substance mg per kg body weight	Coagulation time as per cent of normal before admin.	Coagulation time as per cent of normal after admin.
KOFLER's quinone-45	orally as tablets	279	10	35.85	744	904
	orally as tablets	284	20	70.4	549	556
	orally in ethyl laurate	259	13	50.2	830	1380
	orally in ethyl laurate	423	10	23.65	371	404
	orally in ethyl laurate	433	26	60.0	902	638
	orally in ethyl laurate	446	36	80.8	722	760
KOFLER's quinone-45, semisynthetic	orally in ethyl laurate	423	40	94.6	3000	3000
	orally in ethyl laurate	284	21	74.0	3000	1628
	orally in ethyl laurate	406	84	207	3000	1945
	orally in H_2O with Tween 80	352	80	227	3000	3000
	intravenously in H_2O with Tween 80	650	5	7.7	2333	3000
		770	16	20.8	840	2500
KOFLER's quinone-45, natural, from maple leaves	orally in ethyl laurate	360	37	102.8	840	763
	orally in ethyl laurate	306	61	199.5	457	531
	orally in H_2O with Tween 80	305	140	459	1129	188
Ubiquinone-50	orally as tablets	296	10	33.8	672	807
	orally as tablets	298	20	67.1	204	249
	orally in ethyl laurate	391	20	51.2	873	1000
	orally in ethyl laurate	489	36.8	75.2	524	542
2,3-dimethyl-5-phytyl-benzo-quinone	orally in ethyl laurate	390	20	51.3	650	524
	orally in ethyl laurate	650	52.8	81.2	1457	> 3000
	orally in ethyl laurate	456	30.8	67.5	302	326

In the two cases where KOFLER's quinone was given by intrajugular injection of a colloidal solution the coagulation time increased. It was not possible, however, to administer intravenously such large quantities of the substance as were given orally.

In some cases, especially in the trials with KOFLER's quinone prepared semi-synthetically, chicks with very long coagulation times were used. It is common experience that in simple vitamin K deficiency such extreme coagulation times may fluctuate as indicated in the table without ingestion of test substance.

No significant shortening of coagulation time was observed after ingestion of ubiquinone-50 or 2,3-dimethyl-5-phytyl-benzoquinone.

In the first communication dealing with KOFLER's quinone (1), it was already mentioned that the vitamin K activity of 500 micrograms of this compound is less than that of 1 microgram of menadione.

The fact that ubiquinone is devoid of vitamin K activity was mentioned by MORTON (3) quoting a letter from R. L. LESTER. In the same article, MORTON also mentioned that normal ubiquinone content was found in the livers of rats deficient of vitamin K.

WEINER et al. (4) have shown that in guinea pigs exogenous ubiquinone (Q_{10}) has no vitamin K-like antagonistic action against the hypoprothrombinemia induced by acetocoumarin.

The results of the present tests are in full agreement with these statements. It can, therefore, be concluded that the blood clotting method for assay of vitamin K is not influenced to any important degree by the presence of KOFLER's quinone or ubiquinone.

Summary

KOFLER's quinone with a side chain of 45 carbon atoms, ubiquinone with a side chain of 50 carbon atoms, and a synthetic analogue of KOFLER's quinone, 2,3-dimethyl-5-phytyl-benzoquinone, were assayed for vitamin K activity by the curative blood clotting method in vitamin K deficient chicks. The role of these substances as sources of error in that method was found to be negligible.

Zusammenfassung

KOFLERS pflanzliches Chinon mit Seitenkette von 45 Kohlenstoffatomen, Ubichinon mit Seitenkette von 50 Kohlenstoffatomen, sowie ein synthetisches Analoges von KOFLERS Chinon: 2,3-dimethyl-5-phytyl-benzochinon wurden mittels der kurativen Blutgerinnungsmethode in Vitamin K-defizienten Küken auf Vitamin K-Wirkung untersucht. Es wurde gefunden, daß diese Verbindungen als Fehlerquellen bei der Bestimmung von Vitamin K nach dieser Methode keine Rolle spielen können.

References

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