

14772

Phthalates and Vitamin K.

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Recent publications¹⁻⁵ have suggested that vitamin K activity is due to the eventual conversion of the natural vitamins K₁ and K₂, as well as their synthetic analogues, to phthalic acid or related compounds. According to this theory phthalic acid is regarded as the true biological carrier of vitamin K activity. The evidence for this belief consists largely of the following: (1) reported observations that the highly active 2-methyl-1, 4-naphthoquinone (menadione) and the potassium salt of 2-methyl-1, 4-naphthoquinone-3-sulfonate can be transformed into phthalic acid under mild conditions such as might prevail in the body; and (2) experimental evidence of a slight anti-hemorrhagic activity of phthalic acid and a much higher activity of diethyl phthalate. However, another investigator⁶ could find no evidence of vitamin K activity from the oral administration of 100 mg of potassium acid phthalate or 23 mg of diethyl phthalate, as tested in 300 g vitamin K-deficient chicks. In view of this disagreement in the literature, there may be reported the following investigation on the possible vitamin K activity of several phthalate esters.

Day-old chicks were fed a vitamin K-deficient diet of the heated grain type,⁷ so that

hypoprothrombinemia, as indicated by increased blood coagulation time in several test animals, resulted in 13 to 15 days. The various test substances were administered orally by syringe in 0.1 cc doses. After 18 hours the chicks were bled by decapitation, and blood coagulation time for each chick was determined at room temperature on duplicate samples of approximately 1 cc of blood collected in small vials. The phthalates were of purified chemical grade (Eastman). Cod liver oil (U.S.P.) was used as the solvent in the first experiment and ethyl laurate (Eastman) in the second.

In Table I are given the results of both experiments. A coagulation time of less than 6 minutes was regarded as a positive response for vitamin K activity; in these experiments all of the chicks which received menadione actually had blood coagulation times of ½ to 4 minutes, usually 1 to 2 minutes. A coagulation time of 6 minutes or greater was regarded as a negative response; the coagulation times actually observed in the negative cases were usually above 20 minutes, almost one-half remaining uncoagulated after 1 hour.

As may be seen in the table, no vitamin K activity was shown by dipropyl, di-isopropyl, or dibutyl phthalate at a level of 10 mg per chick, or by diethyl phthalate at levels of 0.1, 10, or 100 mg per chick. On the other hand, a positive response was regularly elicited by 0.01 mg (10γ) or 0.001 mg (1γ) of menadione. Therefore, if diethyl phthalate had any vitamin K activity under the conditions of these experiments, it must have been less than one-hundred-thousandth the potency of menadione. This result is in agreement with the findings of Dam⁶ in larger chicks. It must be noted that the previous reports of the vitamin K activity of diethyl phthalate were apparently based on rat experiments in which hypoprothrombinemia was induced by ligation of the bile duct. Since a different species

¹ Pakendorf, K., Kudryashov, B. A., and Lazareva, E. N., *C. R. acad. sci. U. S. S. R.*, 1941, **31**, 484; *Chem. Ab.*, 1943, **37**, 914.

² Shemiakin, M. M., Schukina, L. A., and Shvezov, J. B., *Nature*, 1943, **151**, 585.

³ Bochar, D. A., Schukina, L. A., Chernyshev, A. S., Semenov, N. G., and Shemiakin, M. M., *J. Am. Chem. Soc.*, 1943, **65**, 2162.

⁴ Shemiakin, M. M., Schukina, L. A., and Shvezov, J. B., *J. Am. Chem. Soc.*, 1943, **65**, 2164.

⁵ Shemiakin, M. M., Shchukina, L. A., and Shvetzov, Yu. B., *J. Gen. Chem. (U. S. S. R.)*, 1943, **13**, 398.

⁶ Dam, H., *Nature*, 1943, **152**, 355.

⁷ Ansbacher, S., *Proc. Soc. Exp. Biol. and Med.*, 1941, **46**, 421.

TABLE I.
Vitamin K Activity as Determined by Oral Administration to Chicks.

	Group	No. chicks	Supplement			Vitamin K activity	
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Exp. I	1	4	Cod liver oil	0.1	cc	4	0
	2	5	Diethyl phthalate	0.1	mg	5	0
	3	5	" "	10.0	"	5	0
	4	4	Dipropyl "	10.0	"	4	0
	5	4	Di-isopropyl "	10.0	"	4	0
	6	4	Dibutyl "	10.0	"	4	0
	7	5	Menadione	0.01	"	0	5
Exp. II	1	5	None			4	1*
	2	6	Ethyl laurate	0.1	cc	6	0
	3	8	Diethyl phthalate	10.0	mg	8	0
	4	9	" "	100.0	"	9	0
	5	8	Menadione	0.001	"	0	8
	6	8	" "	0.01	"	0	8
	7	7	Diethyl phthalate + menadione	0.1 0.001	" "	0	7

* Small chick; had not eaten well.

and a different technic were used in the experiments herein reported, the two investigations are not directly comparable. However, it may be pointed out that oral administration to chicks is the generally accepted method of testing for vitamin K activity. After the completion of these experiments a publication was received in which it was reported⁸ that neither intravenous sodium phthalate nor oral diethyl phthalate increased the prothrombin in a jaundiced man who responded readily to intravenous injection of the phosphoric acid ester of 2-methyl-1, 4-naphthoquinone.

In a recent discussion of the hypoprothrombinemia produced by indandione derivatives,⁹ it was stated that the indandione compounds might degrade to phthalic acid. In this con-

nection may be mentioned the anti-vitamin K or anti-prothrombin test made with diethyl phthalate (Expt. II, group 7). This group of vitamin K-deficient chicks was fed 0.1 mg (100 γ) of diethyl phthalate in ethyl laurate, followed about 15 minutes later by 0.001 mg (1 γ) of menadione. As may be seen in Table I, diethyl phthalate did not prevent the positive response for vitamin K activity from this small dose of menadione under these experimental conditions.

Summary. When tested by oral administration to baby chicks, no vitamin K activity was observed for dipropyl, di-isopropyl, or dibutyl phthalate at a level of 10 mg per chick, or for diethyl phthalate in doses as high as 100 mg per chick. These results differ from those reported in some rat experiments, in which diethyl phthalate was considered active. When tested by a single dose method in depleted chicks, diethyl phthalate did not show anti-vitamin K activity.

⁸ Karrer, P., and Koller, F., *Helvet. Chim. Acta*, 1943, **26**, 2114.

⁹ Kabat, H., Stohman, E. F., and Smith, M. I., *J. Pharm. Exp. Therap.*, 1944, **80**, 160.