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Oral and Parenteral Toxicity of Vitamin K_1, Phthiocol and 2 Methyl 1, 4, Naphthoquinone.

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The antihemorrhagic activity of vitamin K_1 and its homologues has been studied by numerous investigators in animals[1-14] as well as in patients,[15-26] but no systematic investigation of the comparative toxicity of these compounds has as yet been published. Such a study would appear to be particularly indicated in view of the increasing clinical use of some of these compounds.

1 Klose, A. A., Almquist, H. J., and Mecchi, E., *J. Biol. Chem.*, 1938, **125**, 681.

2 Dam, H., and Glavind, J., *Nature*, 1938, **142**, 1077.

3 Murphy, R., *Science*, 1938, **89**, 203.

4 Greaves, J. D., *Am. J. Physiol.*, 1939, **125**, 429.

5 Thayer, S. A., McKee, R. W., Binkley, S. B., MacCorquodale, D. W., and Doisy, E. A., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 478.

6 Ansbacher, S., *J. Nutrition*, 1939, **17**, 303.

7 Almquist, H. J., and Klose, A. A., *J. Am. Chem. Soc.*, 1939, **61**, 1611.

8 Cheney, G., *J. Lab. Clin. Med.*, 1939, **24**, 919.

9 Thayer, S. A., McKee, R. W., Binkley, S. B., MacCorquodale, D. W., and Doisy, E. A., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 194.

10 Almquist, H. J., and Klose, A. A., *J. Am. Chem. Soc.*, 1939, **61**, 1923.

11 Dam, H., and Glavind, J., *Z. Vitaminforsch.*, 1939, **9**, 71.

12 Butt, H. R., Snell, A. M., and Osterberg, A. E., *Proc. Staff Meetings Mayo Clinic*, 1939, **14**, 497.

13 Fernholz, E., Ansbacher, S., *Science*, 1939, **90**, 215.

14 Tishler, M., and Sampson, W. L., *J. Am. Chem. Soc.*, 1939, **61**, 2563.

15 Snell, A. M., Butt, H. R., and Osterberg, A. E., *Am. J. Digestive Diseases*, 1938, **5**, 590.

16 Butt, H. R., Snell, A. M., and Osterberg, A. E., *Proc. Staff Meetings Mayo Clinic*, 1938, **13**, 753.

17 Dam, H., and Glavind, J., *Int. Med. Dig.*, 1939, **34**, 17.

18 Rhoads, J. E., *Surgery*, 1939, **5**, 795.

19 Illingworth, C. F. W., *Lancet*, 1939, **236**, 1031.

20 Scanlon, C. H., Brinkhous, K. M., Warner, E. D., Smith, H. P., and Flynn, J. E., *J. Am. Med. Assn.*, 1939, **112**, 1898.

21 Waddell, W. W., Jr., and Guerry, D., *J. Am. Med. Assn.*, 1939, **112**, 2259.

22 Ivy, A. C., and Gray, J. S., *Surg. Gynecol. Obstet. (Internat. Abst. Surg.)*, 1939, **69**, 1.

23 Zuckerman, I. C., Kogut, B., Jacobi, M., and Cohen, J. Y., *Am. J. Dig. Diseases*, 1939, **6**, 332.

24 Townsend, S. R., and Mills, E. S., *Can. Med. Assn. J.*, 1939, **41**, 111.

25 Olson, K. B., and Menzel, H., *Surgery*, 1939, **6**, 207.

26 Koller, F., and Wuhrmann, F., *Klin. Wchnschr.*, 1939, **18**, 1058.

Acute Toxicity Studies. White mice, weighing approximately 18 to 20 g and 19-day-old chicks, weighing approximately 75 g, were used in these experiments. The compounds being practically insoluble in water, were suspended in peanut oil or sesame oil and administered orally through a blunt metal cannula to a series of 300 mice. To a second series of 240 mice and 100 chicks the drugs were given intraperitoneally in a suspension of sesame oil in order to favor a more complete absorption. In all experiments the amount of solvent given was limited to 0.25 cc of oil per 20 g animal body weight by adjusting the concentration of the suspension. In control experiments, the effect of the solvent was studied by administering peanut and sesame oil in amounts of 0.5 and 1 cc per 20 g animal body weight. Observations were made frequently during the first 5 hours and then once daily for 3 consecutive days.

The results of these experiments are shown in Charts 1 and 2. From these data it can be seen that synthetic vitamin K_1 is considerably less toxic than Phthiocol or 2-methyl-1,4-naphthoquinone when given orally or intraperitoneally. With lethal doses of Phthiocol or 2-methyl-1,4-naphthoquinone mice died usually within the first 5 hours, a few late deaths occurring on the second and third day. In contrast to this, no toxic symptoms were observed with vitamin K_1 in doses up to 25 g per kilo even over a 10-day observation period. However, in view of the fact that mice and chicks injected intraperitoneally with vitamin K_1 and sacrificed after 10 days, still had considerable amounts of the oily suspension in the abdominal cavity, the question must be raised, whether the apparent lack of toxicity of vitamin K_1 is not at least in part due to an extremely slow rate of absorption.

CHART I.

Acute Oral Toxicity of 2-methyl-1,4-naphthoquinone, Phthiocol and Vitamin K_1 in Mice.

Dose, g/kg	2-methyl-1,4-naphthoquinone	Phthiocol	Vitamin K
	% mortality		
0.100	0		
0.200	0	20	
0.300	—	70	
0.400	35	80	
0.600	50	100	
0.800	95	—	
1.000	100	—	
1.200	100	—	
1.500			
15.00			0
20.00			0
25.00			0

Total of 300 animals; 20 mice per dose level.

CHART II.

Intraperitoneal Toxicity of 2-methyl-1,4-naphthoquinone, Phthiocol and Vitamin K_1 in Mice.

Dose, g/kg	2-methyl-1,4-naphtho-quinone	Phthiocol	Vitamin K_1
	% mortality		
0.050	10	0	
0.075	50	0	
0.100	90	0	
0.150	95	30	
0.200	100	100	
0.250	100	100	
0.350			
0.600			
1.000			
15.00			0
20.00			0
25.00			0

Total 240 animals, 20 mice per dose level.

Intraperitoneal Toxicity of 2-methyl-1,4-naphthoquinone, Phthiocol and Vitamin K_1 in Chicks.

Dose, g/kg	2-methyl-1,4-naphtho-quinone	Phthiocol	Vitamin K_1
	% mortality		
0.10	70	50	
0.15	90	90	
0.25	100	100	
0.50	100	100	
1.00			
25.00			0

Total 100 chicks; 10 chicks per dose level on 2-methyl-1,4-naphthoquinone and Phthiocol and 20 chicks on vitamin K_1.

Chronic Toxicity. The effects of daily oral administration of vitamin K_1, Phthiocol and 2-methyl-1,4-naphthoquinone were studied in 90 young rats by feeding varying amounts of these compounds over a period of 30 consecutive days. The drugs were suspended in 10% gum acacia and doses of 0.1 and 0.35 g per kg of Phthiocol; 0.25, 0.35 and 0.5 g per kg of 2-methyl-1,4-naphthoquinone; and 0.35 and 2 g per kg of vitamin K_1 were administered by stomach tube. Gum acacia was employed in these experiments for the preparation of the suspension in order to avoid the cathartic effect likely to result from prolonged administration of oil. The weight of the animals was recorded daily and the blood picture taken in weekly intervals.

There was no significant effect upon the growth curve with any of these compounds. Rats on doses of 0.1 g per kg of Phthiocol, 0.35 g per kg of 2-methyl-1,4-naphthoquinone and 2 g per kg of vitamin K_1 appeared to remain normal whereas doses of 0.35 g per kg of Phthiocol and 0.5 g per kg of 2-methyl-1,4-naphthoquinone were lethal. Most of the animals fed toxic doses of Phthiocol died during

the first few days of the experiment while the animals fed 2-methyl-1,4-naphthoquinone died on various days over the 30-day feeding period. A marked fall of the erythrocyte count and hemoglobin was observed in rats fed doses of 0.1 g per kg of Phthiocol, and 0.35 g per kg of 2-methyl-1,4-naphthoquinone while vitamin K_1 failed to produce such an effect.

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Summary. The acute and chronic toxicity of Phthiocol, 2-methyl-1,4-naphthoquinone and vitamin K_1 was studied in mice, rats, and chicks. The oral L.D. 50 in mice was found to be approximately 0.2 g per kg for Phthiocol and 0.5 g per kg for 2-methyl-1,4-naphthoquinone; no lethal effect could be produced by doses up to 25 g per kg of vitamin K_1. In the chronic experiments in rats, daily feeding over a period of 30 consecutive days of 0.35 g per kg of Phthiocol, and 0.5 g per kg of 2-methyl-1,4-naphthoquinone was toxic; doses of 0.1 g per kg of Phthiocol and 0.35 g per kg of 2-methyl-1,4-naphthoquinone produced a marked fall of the erythrocyte count and hemoglobin. No such effects were observed following vitamin K_1 administration. In the abdominal cavity of animals sacrificed 10 days after an intraperitoneal injection of vitamin K_1 considerable amounts of an oily suspension could be observed, indicating an extremely slow rate of absorption of vitamin K_1.

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Effects of Culture Filtrates and Old Medium on Growth of the Ciliate, *Colpidium campylum.*

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Effects of protozoan metabolic products on growth of homologous species have been investigated by several workers. One view, based originally upon Woodruff's[1] findings, predicates that waste products of a given species exert an inhibitory effect on growth of that species. The opposite view is represented by Dimitrowa's[2] conclusion that growth of *Paramecium caudatum* is accelerated by small amounts of old culture fluid added to fresh cultures. Johnson and Hardin[3]

1 Woodruff, L. L., *J. Exp. Zool.*, 1911, **10**, 557.

2 Dimitrowa, A., *Zool. Anz.*, 1932, **100**, 127.

3 Johnson, W. H., and Hardin, G., *Physiol. Zool.*, 1938, **11**, 333.