hypothesis formulated by the other workers that the sex hormones act through the pituitary to produce typical changes in the breast is a likely possibility for at least a part of the changes.

We have no explanation for the wide variations in weight in the hypophysectomized animals which were treated at the same time with identical doses of growth complex and estradiol benzoate. Although no microscopic examination was made, the pituitary fossa was examined carefully at autopsy in each instance for evidence of fragments of the anterior pituitary gland. As has been stated above, many of the animals gained or maintained their initial weight after the administration of growth complex. After about 3 weeks, however, they commenced to lose weight rather rapidly. It is reasonable to assume, therefore, that the hypophysis was effectively removed. Several possibilities suggest themselves: (a) The large doses of estrogen may be toxic to the animal; (b) estrogens may inhibit the action of the growth hormone; (c) the animal may become refractory to growth complex. In another experiment we have evidence that the second view is the more tenable. This is also fortified by the work of Zondek, who was able to stunt the growth of immature mice by injecting rather large doses of estrogenic hormone, which probably reduced the anterior pituitary secretion. Experiments to clarify these points are being carried out at present by using much smaller doses of estrogen in combination with the same dose of growth complex.

Conclusion. Nutrition of the animal is an important factor in the effects noted on the breasts of hypophysectomized rats after injection of estrogenic hormone. It is probable, however, that this hormone exerts a part of its effect on the breast by way of the hypophysis.

11008

Natural Vitamin K and Synthetic Vitamin K_1.

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Several investigators[1-4] described in preliminary form the synthesis of 2-methyl-3-phytyl-1,4-naphthoquinone and its identification with vitamin K_1. In regard to their respective biological ac-

[1] Almquist, H. J., and Klose, A. A., *J. Am. Chem. Soc.*, 1939, **61**, 2557.

[2] Binkley, S. B., Cheney, L. C., Holcomb, W. F., McKee, R. W., Thayer, S. A., MacCorquodale, D. W., and Doisy, E. A., *J. Am. Chem. Soc.*, 1939, **61**, 2558.

[3] Fieser, L. F., *J. Am. Chem. Soc.*, 1939, **61**, 2559.

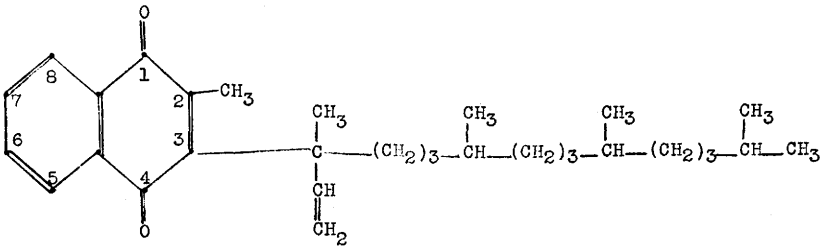
[4] Fieser, L. F., *J. Am. Chem. Soc.*, 1939, **61**, 2561.

tivities, general statements have been made to the effect that both compounds have equal potency.[3-6]

We have prepared 2-methyl-3-phytyl-1,4-naphthoquinone by a modified Fieser[3] procedure with final purification by chromatographic adsorption on calcium sulfate. Using the method of Claisen and Eisleb,[7] we synthesized an isomeric compound, 2-methyl-3-"isophytyl"-1,4-naphthoquinone (Formula I). The biological assays of these compounds are shown in Table I together with those of the Synthetic Vitamin K_1 (Merck),* of the 2-methyl-1,4-naphthoquinone and the diacetate of the corresponding hydroquinone, and of the diacetate of the 2-methyl-3-phytyl-1,4-naphthohydroquinone. The data presented permit the conclusion that the 2-methyl-3-phytyl-1,4-naphthoquinone has a potency of 1 unit[8] in about 7½ γ, its diacetate derivative 1 unit in approximately 15 γ. Furthermore, the Synthetic Vitamin K_1 (Merck), the isomeric compound and 2-methyl-3-dihydrophytyl-1,4-naphthoquinone showed 1 unit in about 15 γ, whereas the methylnaphthoquinone and the diacetate of its corresponding hydroquinone had 1 unit in about ½ γ and 1γ, respectively, as already reported.[9]

The phytyl derivatives appear to have considerably more potency, when the test period is prolonged to 18 hours, as shown in Table II; The speed of action of the unit of 2-methyl-1,4-naphthoquinone is practically identical with that of the unit of the natural vitamin K. However, the phytyl derivatives are not as rapidly utilized by the animal body, as a comparison of Table I with Table II and Table III shows.

FORMULA I.



5 MacCorquodale, D. W., McKee, R. W., Binkley, S. B., Cheney, L. C., Holcomb, W. F., Thayer, S. A., and Doisy, E. A., *J. Biol. Chem.* 1939, **130**, 433.

6 Almquist, H. J., and Klose, A. A., *J. Biol. Chem.*, 1939, **130**, 791.

7 Claisen, L., and Eisleb, O., *Ann.*, 1913, **401**, 21.

* We wish to take this opportunity of thanking Dr. W. L. Sampson of the Merck Institute for Therapeutic Research for his kindness in sending a sample of Synthetic Vitamin K_1 (Merck).

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

9 Fernholz, E., and Ansbacher, S., *Science*, 1939, **90**, 215.

TABLE I.
Biological Assays.

Dose, γ	No. of chicks	Blood clotting time (minutes) Before treatment (ave)	6 hours after treatment (individual chicks)								
		2-Methyl-3-phytyl-1,4-naphthoquinone									
10	5	>90	<1	<1	<2	<2	<2				
7½	10	>90	<2	<2	<3	<4	<4	<5	<5	<6	<8	<10
5	10	>90	<3	<3	<4	<4	<5	<15	<15	>30	>30	>30
		2-Methyl-3-"isophytyl"-1,4-naphthoquinone									
15	10	>90	<2	<2	<3	<3	<3	<3	<5	<8	<10	<12
10	9	>90	<2	<2	<4	<8	<10	>30	>30	>30	>30	
		Synthetic Vitamin K_1 (Merck)									
15	10	>90	<2	<3	<4	<4	<4	<5	<6	<10	<10	<12
10	10	>90	<3	<4	<5	<5	<7	<7	<10	<21	>30	>30
5	8	>90	<6	<6	<10	<13	>30	>30	>30	>30		
		2-Methyl-3-phytyl-1,4-naphthohydroquinone-diacetate									
15	8	>90	<3	<3	<4	<5	<5	<8	<11	<14		
10	10	>90	<3	<3	<4	<6	<9	<13	<15	>30	>30	>30
		2-Methyl-1,4-naphthoquinone									
½	10	>90	<2	<2	<2	<2	<3	<3	<4	<5	<5	<6
¼	10	>90	<2	<2	<3	<3	<4	<6	<7	<22	>30	>30
		2-Methyl-1,4-naphthohydroquinone-diacetate									
1	10	>90	<2	<2	<3	<3	<4	<5	<5	<5	<8	<13
¾	10	>90	<2	<3	<3	<4	<6	<6	<7	<24	>30	>30

These biological data are particularly interesting, when one considers that we have obtained repeatedly vitamin K concentrates from alfalfa with potencies of 1 unit in about 2 γ,[10] and that these preparations gave a Dam-Karrer color reaction[11, 12] not nearly as strong as the one of 2-methyl-3-phytyl-1,4-naphthoquinone. It seems evident that a vitamin K different from vitamin K_1 exists in alfalfa.

In our experience, concentrates prepared from alfalfa and with a

TABLE II.
Curative Effect

	2-Methyl-3-phytyl-1,4-naphthoquinone 2γ				2-Methyl-3-"Isophytyl"-1,4-naphthoquinone 2γ				Synthetic Vitamin K_1 (Merck) 2γ			
	Blood clotting time (min) of individual chicks after											
Hours	0	6	18	48	0	6	18	48	0	6	18	48
	>90	<5	<1	<2	>90	<6	<2	<10	>90	<4	<1	<3
	>90	<5	<1	<12	>90	>30	<2	<4	>90	<8	<1	>30
	>90	>30	<2	<3	>90	>30	<2	<4	>90	<12	<1	<5
	>90	>30	<2	<6	>90	>30	<2	<7	>90	>30	<2	<3
	>90	>30	<2	>30	>90	>30	<2	>30	>90	>30	<2	<21

[10] Fernholz, E., Ansbacher, S., and Moore, M. L., *J. Am. Chem. Soc.*, 1939, **61**, 1613.

[11] Dam, H., Geiger, A., Glavind, J., Karrer, P., Karrer, W., Rothschild, E., and Salomon, H., *Helv. Chim. Acta*, 1939, **22**, 310

[12] Karrer, P., *Helv. Chim. Acta*, 1939, **22**, 1146.

TABLE III.
Minimum Effective Doses in 6- and 18-hour Tests.

Substance	6 hr	18 hr	Ratio
	γ	γ	
Vitamin K_1	15	1	15:1
Methylnaphthoquinone	½	¼	2:1
Alfalfa Concentrate	2	1	2:1

potency of 1 unit in about 30 γ are quite complex in composition and contain several chemical individuals with varying vitamin K potencies, as investigations by chromatographic analyses on calcium sulfate show. Some of these fractions are deep red, others are yellow, but our most potent concentrates were always nearly colorless. It should be noted that our process of isolation is different from that employed by other investigators and this may be one of the reasons why we were able to obtain concentrates which had such a high potency but did not give the typical color reaction for vitamin K_1.

11009

Nitrogen Retention on Casein Digests.

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Early work with digests of casein showed that nitrogen retention and growth in young dogs and rats might be promoted[1, 2] by digests prepared by the combined action of digestive juices. In these studies and in those of Hopkins[3] it was recognized that acid digests were incomplete mixtures and had to be supplemented by the addition of tryptophane. Not all workers have been able to confirm these original findings.[4] This may have been due to the quality of the hydrolysates employed. Enzymic hydrolysis, according to Abderhalden's procedure, involves the use of gastric, pancreatic and intestinal extracts, and digestion for a considerable period of time. Boiling hydrochloric or sulfuric acid of the concentration ordinarily used for acid hydrolysis may result in the formation of by-products (amines?) which, in the absence of adequate purification procedures, may mask the actual nutritive value of the digest. A purified casein digest prepared with

1 Abderhalden, E., and Oppler, B., *Z. physiol. Chem.*, 1907, **51**, 226; *J. C. S.*, 1907, ii A, 369.

2 Abderhalden, E., *Z. physiol. Chem.*, 1915, **96**, 1.

3 Hopkins, F. G., *J. Chem. Soc.*, 1916, **109**, 629.

4 McClendon, J. F., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 915.