

TABLE II.
Subcutaneous Toxicity in Mice.

Compound	Dose, mg/kg	Mice died/Mice used
IX	250	2/13
	300	8/10
	350	9/10
	400	10/10
	500	10/10
Cocaine	75	2/10
	100	4/10
	150	9/10

to produce local anesthesia in 1.0% and 0.5% solution but all caused tissue damage at the site of injection. In 1.0% solution certain of

these derivatives abolished the corneal reflex after instillation in rabbit eyes. The hydrochloride of (2-piperidinomethyl-) 4,6-dimethylphenyl *p*-aminobenzoate (IX) was less toxic subcutaneously in mice and exhibited greater topical activity in rabbits than cocaine hydrochloride but practical application of the anesthetic action of IX or any of the new compounds is complicated by their instability in aqueous solution.

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Antihemorrhagic Vitamin Effect of Honey.*

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While working on the role of honey in the prevention and cure of nutritional anemia in rats, one of the authors was impressed by the fact that the coagulability of blood in the rats receiving a honey supplement was so high that it was sometimes extremely difficult to draw samples for the hemoglobin determinations. The natural antihemorrhagic vitamins K are fat soluble substances. However, Warner and Flynn¹ have shown that there are water soluble substances which show antihemorrhagic activity. Therefore, it was considered advisable to test honey for the presence of factors influencing the coagulability of blood.

The method followed was a slight modification of the vitamin K assay devised by Almquist² using chicks. Herring meal was used in the basal diet instead of sardine meal and the temperature of the thermostat in which

coagulability was determined was 37°C instead of 38°-39°C. In this procedure the only variable is the prothrombin level of the blood. Under these conditions the coagulability is referred to as "prothrombin time." Almquist² has found that a direct proportionality exists between $10 \times$ (reciprocal of the prothrombin time, in seconds) and the log ($10 \times$ vitamin K intake).

Three series of experiments were performed, shown in Tables I, II and III. These were carried out at different times using 3 different but fresh preparations of thromboplastin for determination of prothrombin time. Each series required a different set of normal and negative controls.

In the first series, shown in Table I, mixed sweet clover and white clover honey, alfalfa and buckwheat honey were tested. The first 2 were mixed with the vitamin deficient ration in the ratio of 1:4 by weight; the buckwheat honey was added to the ration in the ratio of 1:3. The supplements were offered for 4 days to the vitamin K deficient chicks after the depletion period. The control chicks were given a solution of 2-methyl-1,4 naphtho-

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¹ Warner, E. D., and Flynn, J. E., *Proc. Soc. Exp. Biol. and Med.*, 1940, **44**, 607.

² Almquist, H. J., *J. Assn. Off. Agr. Chem.*, 1941, **24**, 405.

TABLE I.
Antihemorrhagic Vitamin Effect of Honey.

Diet	Total supplement (4 days)	No. of chicks	Prothrombin time	
			Mean and standard error, sec.	Coeff. of variability, %
Normal diet	—	5	44 ± 1.0	4.7
Basal + 0.5 µg stand. vit. K in ethyl laurate (once daily)	2 µg	7	237 ± 4.6	4.7
Basal + 2.0 µg stand. vit. K in ethyl laurate (once daily)	8 "	8	59 ± 1.9	8.3
Basal containing 20% mixed honey	8 g	15	210 ± 48.0	85.8
Basal containing 25% buckwheat honey	10 "	12	173 ± 3.3	8.4
Basal containing 20% alfalfa honey	8 "	14	228 ± 3.9	6.3
Basal only	—	6	>1 hr	—

TABLE II.
Effect on Prothrombin Time of Administering Standard Vitamin K in Water vs. in Ethyl Laurate.

Diet	Total supplement (4 days)	No. of chicks	Prothrombin time	
			Mean and standard error, sec.	Coeff. of variability, %
Normal diet	—	5	49 ± 1.3	5.3
Basal + 2 ml aqueous susp. vitamin K nearly equal to 3 µg (once daily)	12 µg	20	157 ± 5.2	16.1
Basal + 3 µg stand. vit. K in ethyl laurate (once daily)	12 "	10	51 ± 0.5	2.8
Basal only	—	7	>1 hr	—

TABLE III.
Lack of Vitamin K Activity of Alfalfa Hay and Honey When Administered in Water Suspension and Restoration of Normal Effect by Giving Ethyl Laurate.

Diet	Total supplement (4 days)	No. of chicks	Prothrombin time	
			Mean and standard error, sec.	Coeff. of variability, %
Normal diet	—	7	40 ± 1.0	6.4
Basal + 0.5 µg stand. vit. K in ethyl laurate (once daily)	2 µg	5	176 ± 3.8	4.4
Basal + 2 µg stand. vit. K in ethyl laurate (once daily)	8	6	48 ± 1.3	5.9
Basal + 2 ml 10% susp. alfalfa hay (once daily)	8	7	dead of hemorrhage	
Basal + 2 ml 10% susp. alfalfa hay + 2 drops Et. laurate (once daily)	8	9	126 ± 4.5	10.1
Basal + 2 ml 50% sol. alf. honey (3 times daily)	12	8	1686 ± 396	61.7
Basal + 2 ml 50% sol. mixed honey (3 times daily)	12	5	972 ± 108	21.8
Basal + 2 ml 50% sol. buckwheat honey (3 times daily)	12	6	912 ± 66	17.1
Basal only	—	6	>1 hr	—

quinone in ethyl laurate orally by means of pipette, and were not allowed any access to the food and water for half an hour after dosing.

Assuming the proportionality between prothrombin time and vitamin K intake found by Almquist, it may be calculated from graphs constructed from our results that the honeys tested when mixed with a vitamin K deficient ration showed a definite antihemorrhagic activity equivalent to approximately 0.25 μ g of 2-methyl-1,4 naphthoquinone per gram of honey. However, when an aqueous solution of honey was administered directly to vitamin K depleted chicks, the prothrombin time was considerably greater. The actual dilution employed was not recorded and no attempt made to determine actual clotting time. This result was so surprising that it seemed to warrant further study.

It is known that the bile favors the utilization of vitamin K.^{3,4} The suggestion was therefore advanced that when an aqueous solution of honey is administered to chicks normal bile secretion does not occur, thus preventing an adequate absorption of the antihemorrhagic vitamin. To test this hypothesis, 2-methyl-1,4 naphthoquinone was administered by pipette to vitamin K deficient chicks in aqueous suspension with and without the addition of ethyl laurate. The results presented in Table II show that a direct feeding of vitamin K in a water suspension increased the prothrombin time about 3 times over that of the normal controls or when the vitamin was administered in ethyl laurate.

Since alfalfa hay is known to be a good source of vitamin K a further study of the type of dosing procedure was made by comparing the effect of administering this material in aqueous suspension with and without ethyl laurate with the effect of the standard vitamin

K with ethyl laurate. A further study was also made at this time of administering a solution of 3 different types of honey, concentrated, *i.e.*, 50%, solutions being used. The results given in Table III show that the chicks fed alfalfa hay in aqueous suspension died of hemorrhage while an addition of only 2 drops of ethyl laurate to this suspension brought the prothrombin time to about 3 times the normal. Graphs constructed from the results of the tests with the standard vitamin in series A and C, Tables I and III, correlating the reciprocal of the prothrombin time with the log vitamin dose, give closely parallel lines. The tests with honey diluted with equal volume of water administered directly to the prothrombin deficient chicks resulted in little evidence of vitamin K activity, confirming the results of the previous test cited in connection with series A. The clotting times observed are outside of the range of correct assay of vitamin K by this procedure.

Summary. Buckwheat, alfalfa and mixed honeys when fed incorporated in basal ration to vitamin K depleted chicks were found to possess a definite antihemorrhagic activity equal to approximately 0.25 μ g of 2-methyl-1,4 naphthoquinone per gram of honey. The antihemorrhagic activity of honey was greatly lowered when honey was administered in an aqueous solution directly to the vitamin K depleted chicks.

It was demonstrated that the antihemorrhagic activity of 2-methyl-1,4 naphthoquinone is reduced about 3 times when fed in aqueous suspension. The antihemorrhagic activity of alfalfa hay in water suspension was practically nil while with an addition of 2 drops ethyl laurate, the prothrombin time was quite satisfactory.

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³ Greaves, J. D., and Schmidt, C. L. A., *Proc. Soc. Exp. Biol. and Med.*, 1937, **37**, 43.

⁴ Dam, H., and Glavind, J., *Acta Medica Scand.*, 1938, **96**, 108.