

color, if sufficient vitamin K is present and interfering pigments are practically absent. Soon the color changes to a reddish-purple and finally to a reddish-brown. At this point, carotenoid pigments may be removed by partition with a hydrocarbon solvent. The color due to reaction of the vitamin with sodium methylate remains in the methanol phase. To test the agreement of color reaction with activity, we have applied this reaction to a variety of sources of the vitamin assayed by a procedure already described [*Biochem. J.*, **32**, 1897 (1938)]. Results are given in Table I.

Fractions obtained by chromatographic adsorption showed a consistent relation of color test to activity. This was also true of fractions obtained by incomplete molecular distillation and of a preparation (concentrate 1270) obtained by repeated precipitation from methanol by chilling with solid carbon dioxide [*J. Biol. Chem.*, **120**, 635 (1937)] but not purified from sterols. A preparation of the molecular compound of the vitamin with deoxycholic acid [THIS JOURNAL, **61**, 745 (1939)] showed a color reaction consistent with its activity, which was also true of the residue of this preparation remaining after partial extraction of the vitamin with xylene. A strong color reaction was also produced on testing an active concentrate prepared by repeated molecular distillation of soybean oil, followed by removal

of sterols, free fatty acids and waxes. In addition to the data in the table, we may report that the color reaction has been obtained in extracts of several kinds of bacteria known to be good sources of the vitamin [*Proc. Soc. Exp. Biol. Med.*, **38**, 336 (1938)].

The results strongly indicate that the color reaction is due to the vitamin itself. The character of the pigment is being studied further.

DIVISION OF POULTRY HUSBANDRY H. J. ALMQUIST
DEPARTMENT OF AGRICULTURE A. A. KLOSE
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIF.

RECEIVED MAY 19, 1939

THE ANTI-HEMORRHAGIC ACTIVITY OF PURE SYNTHETIC PHTHIOL

Sir:

We wish to announce the discovery of the anti-hemorrhagic activity of pure synthetic pthiol, 2-methyl-3-hydroxy-1,4-naphthoquinone. The physical and chemical properties of this compound are in general similar to those known for vitamin K. When fed to chicks at a level of 20 mg. per kg. of vitamin K-free diet, pthiol maintained the average blood-clotting time at 2.1 minutes in one test and 1.6 minutes in a second test. At a level of 10 mg. the blood-clotting time was maintained at 1.8 minutes. Chicks fed only the basal ration had prolonged blood-clotting times. The minimum required level is being determined. It is probable that pthiol is the simplest member of an homologous series of anti-hemorrhagic substances.

We are indebted to Professor R. J. Anderson for the pthiol used in these experiments.

DIVISION OF POULTRY HUSBANDRY H. J. ALMQUIST
DEPARTMENT OF AGRICULTURE A. A. KLOSE
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIF.

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CHROMATOGRAPHIC ADSORPTION AND DIPOLES

Sir:

The use of the method of chromatographic adsorption has become extremely important for the separation of complex mixtures of organic molecules.

A careful survey of numerous experimental investigations has revealed the importance of dipoles in determining the order of adsorption of a mixture on a polar medium (*i. e.*, aluminum oxide). Thus Karrer [Karrer and Njelsen, *Ber.*

TABLE I
ANTHEMORRHAGIC ACTIVITY AND COLOR REACTION
INTENSITY OF VITAMIN K CONCENTRATES

Concentrate	Level fed per kilo of diet, mg.	Average blood clotting time, min.	Relative intensity of color test
Chromatographic adsorption fractions			
1 Orange zone	10	2.8	4
2 Light yellow zone	10	1.8	8
3 Yellow zone	10	6.3	2
4 Colorless zone	10	>30	0
Incomplete distillation fractions			
1 Low temp. distillate, P11	80	>30	0
2 Vitamin distillate, P11	20	4.2	2
3 Residue, P11	20	7.3	1
4 Vitamin distillate, P8	10	3.5	4
5 Residue, P8	10	3.7	4
6 Vitamin distillate, P12	20	3.7	2
Other preparations			
1 Concentrate 1270	10	4.7	4
2 K-choleic acid, 8P	30	2.7	4
3 K-choleic acid, 8P xylene extracted	30	14.1	1
4 Soybean oil concentrate	400	3.0	strong