boundary at that point. Throughout the experiment the boundary remained single and sharp, exhibiting no evidence of splitting. With continuous migration, slight spreading of the boundary occurred due to combined influence of diffusion and inhomogeneity of particle charge. At 0°C diffusion of the large molecules is very small and most of the spread is attributable to the latter factor. The extreme sharpness of the last single peak of Fig. 1 shows a remarkably high degree of uniformity of particle charge, indicative of corresponding homogeneity of the papilloma-virus protein in the acid range below the isoelectric point. Preliminary studies have been made in the region of solubility above the isoelectric point. Here, the protein moved with a single sharp boundary with slightly more spread and skewness in the final picture than in that of Fig. 1. In none of the studies thus far made (between pH 3.78 and pH 7.7) has there been any evidence of boundary splitting.

The results show that the papilloma protein is extremely well suited to studies in the Tiselius apparatus. Under the conditions described solutions of this animal virus protein exhibit on both sides of the isoelectric point a high degree of electrophoretic homogeneity comparable to or possibly greater than that of the most homogeneous hemocyanins[9] and crystalline proteins.[10] These findings combined with those of sedimentation studies provide a valuable criterion of the physical state of the material since the degree of homogeneity observed with the papilloma protein has been seen thus far only with solutions of a single molecular species.

12013

Additional Observations on Vitamin K-Deficient Diets.

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In recent papers Almquist[1, 2] emphasizes the precautions necessary in the care and housing of test animals in order to prevent bacterial

[9] Tiselius, A., and Horsfall, F., Jr., *Arch. f. Kemi, Mineralogi och Geologi*, 1939, **13** A, 1.

[10] Longsworth, L. G., Cannan, R. K., and MacInnes, D. A., *J. Am. Chem. Soc.*, 1940, **62**, 2580.

* Present address: International Vitamin Corporation, 50 East 42nd Street, New York City.

K-vitamin synthesis in wet feeds and in droppings, and states that heated diets[3] are not growth-promoting. These reports prompt us to present additional data on this type of diets.

Experiments conducted during the past months have shown that ration K-11 (Table I) containing p-aminobenzoic acid[4] is more suitable than the ration K-7, previously described[3] as being deficient in the rat anti-gray-hair factor, because it permits better growth of the test animals and an earlier incidence of the hemorrhagic diathesis. It was observed also that vitamin K is apparently a growth factor, since chicks lose weight when the deficient symptoms occur and grow at a greater rate on ration K-11 supplemented with vitamin K than on ration K-11 alone.

The type of casein in ration K-11 is an important factor. The SMACO† Vitamin-free Casein proved to be an excellent source of protein in a vitamin K-deficient diet, but Labco‡ Vitamin-free

TABLE I.
Vitamin K-deficient Ration K-11.*

			%
Heated grain mixture†	wheat middlings	25	83
	yellow corn	58	
Casein‡			12
Salt mixture[5]			2
Calcium carbonate			1
Cod liver oil (medicinal)			2

*Contains less than 0.1 μg of biotin per g and should, therefore, be supplemented with biotin, when the animals are not to be sacrificed at the termination of the vitamin K studies. Without added biotin, most of the chicks showed severe vitamin H-deficiency symptoms within 2 months and perosis was observed in about 20% of the birds at the end of the third month.

†A mixture containing 25 parts of wheat middlings and 58 parts of yellow corn heated to 120°C for one week.

‡A dilute ethanol solution containing

80 mg { thiamine hydrochloride, riboflavin, pyridoxine hydrochloride }
+
1 g { calcium pantothenate, nicotinic acid, inositol }
+
3 g { choline chloride, p-aminobenzoic acid }

is added to 1.2 kg of SMACO Vitamin-free Casein; the product is thoroughly mixed and then dried at a temperature not exceeding 70°C.

1 Almquist, H. J., 56th Ann. Meet., A.O.A.C., Washington, D.C., October, 1940.

2 Almquist, H. J., *Physiol. Rev.*, 1941, **21**, 194.

3 Ansbacher, S., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 248.

4 Ansbacher, S., *Science*, 1941, **93**, 164.

† Distributed by the General Biochemicals, Inc., Chagrin Falls, Ohio.

‡ Distributed by the Borden Co., New York.

Casein is apparently not entirely devoid of vitamin K, as the hemorrhagic diathesis does not develop in all the test animals reared on this diet in which this brand of casein is used as the basal protein. A similar observation has already been made in a comparative study of rations containing the Labco product and fish meal, respectively.[5]

The ration K-11 can be used even when wet. One hundred day-old chicks were placed on a diet consisting of ration K-11 thoroughly mixed with an equal part of fresh ripe bananas. All the birds on this diet showed the typical K-deficiency symptoms within the same period of time as the control birds on ration K-11. Apparently, bananas are devoid of vitamin K and do not contribute to a K-vitamin synthesis. This experiment shows also that ration K-11 is suitable for prophylactic bioassays necessary for the studies of substances with too low a vitamin K content for curative tests.

Every animal of 5 lots of 200 chicks each reared on ration K-11 became sufficiently vitamin K-deficient to be used for assays. The results obtained with some of these birds are recorded in Table II. The "Thyloquinone"§ employed was a corn oil solution containing 1 mg of 2-methyl-1,4-naphthoquinone per ml and the "Thylohydroquinone"§ was an isotonic solution containing 1 mg of 2-methyl-1,4-naphthohydroquinone per ml which had been stored in sealed ampoules at 105°F for a period of 3 months. The "Phosphate (I)" was the previously described product[6] and the "Phosphate (II)" was the preparation "N-123" of the Laboratories of Hoffmann-La Roche,‖ both phosphates presumably being sodium 2-methyl-1,4-naphthohydroquinone diphosphate.[7] The "Amino (2)" and the "Amino (3)" were the 2-methyl-4-amino-1-naphthol hydrochloride[8] and the 3-methyl-3-amino-1-naphthol hydrochloride,[8] respectively, both prepared by Parke, Davis Laboratories.¶ The "Quinoline-quinone" was the new compound 6-methyl-5,8-quinolinequinone recently described by Christiansen and Dolliver.[9]

The data of Table II confirm our previous results[6] that methyl-

[5] Ansbacher, S., *J. Nutrition*, 1939, **17**, 303.

§ Distributed by E. R. Squibb & Sons, New York.

[6] Ansbacher, S., Fernholz, E., and Dolliver, M. A., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 652.

‖ We wish to take this opportunity of thanking Dr. Foster for his kindness in sending us this preparation.

[7] Lee, J., Solmssen, U. V., Steyermark, A., and Foster, R. H. K., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 407.

[8] Emmett, A. D., Kamm, O., and Sharp, E. A., *J. Biol. Chem.*, 1940, **133**, 285.

¶ We wish to take this opportunity of thanking Dr. Kamm for his kindness in sending us these preparations.

[9] Christiansen, W. G., and Dolliver, M. A., *J. Am. Chem. Soc.*, 1941, **63**, in press.

TABLE II.
Vitamin K Activity Determined with Chicks on Ration K-11.

Substance	No. of animals used	Minimum effective dose in 6-hr test γ
Thyloquinone	154	½
Thylohydroquinone	233	½
Phosphate (I)	59	10
" (II)	23	1½
Amino (2)	26	1¼
" (3)	35	2
Quinoline-quinone	31	10 mg insufficient
Bananas (fresh, ripe)*	100	4 g daily insufficient

*Prophylactic test.

naphthoquinone and methylnaphthohydroquinone have identical biological activities. Apparently, solutions of the latter compound are stable even at elevated temperatures. In regard to the discrepancy in the potencies of the 2 phosphate derivatives, the Hoffmann-LaRoche preparation (Phosphate II) was found to be about as potent as methylnaphthoquinone on a molecular basis, thus confirming the recent data of Fieser, *et al.*[10] As suggested by Lee, *et al.*,[7] it may be that the Phosphate (I) had a low activity due to the fact that its preparation did not include the isolation and purification of the intermediate diphosphoryl chloride. The observation of Emmett, *et al.*,[8] that the amino compound with the methyl group in position 2 is more potent than the one with the methyl group in position 3, was confirmed. It is of interest to note also that the quinoline-quinone has apparently no antihemorrhagic activity.

12014

Blood Prothrombin Levels and Hippuric Acid Excretion Liver Function Test in Liver Disease.

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Wilson[1] showed that a close correlation existed between the hippuric acid excretion and the plasma prothrombin levels of 36 patients

10 Fieser, L. F., Tishler, M., and Sampson, W. L., *J. Biol. Chem.*, 1941, **137**, 659.
1 Wilson, S. J., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 559.