These experiments appear to confirm the view, now held by several investigators besides ourselves,[3] that globulins may occur in complexes, easily resolved into their components. In this instance the resolution has been effected by applying a sufficient potential gradient.

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Assay of Vitamin K Concentrates.*

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Since our extensive experience in the assay of estrogens has led to an appreciation of the importance of the contribution of Trevan[1] to bioassay, our work on the assay of Vitamin K was directed toward the establishment of standard curves of response to the administration of the vitamin. Based on Trevan's principles a curative method of assay which appears to have certain advantages over methods previously used by Schønheyder,[2] Almquist and Stokstad,[3] and Almquist, Mecchi and Klose[4] has been developed.

Experimental Diet. In our earlier work, difficulty in consistently producing the deficiency was encountered. It seems that this was due chiefly to diets that were not entirely devoid of Vitamin K. The diet now used is one described by Almquist.[5] Its composition is: fish meal, 17.5 parts; dried brewer's yeast, 7.5 parts; ground polished rice, 73 parts; sodium chloride plus small amounts of cupric and ferrous sulfates, 1.0 part; and cod liver oil 1.0 part. The fish meal and yeast were extracted with hot isopropyl ether before incorporation in the diet.

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3 Jameson, E., *Symposia on Quantitative Biology*, Cold Spring Harbor Biological Lab., 1938, **6**, 331; Sørensen, S. P. L., *Kolloid Z.*, 1930, **53**, 102; Kendall, F. E., *J. Clinical Invest.*, 1937, **16**, 921; McFarlane, A. S., *Biochemical J.*, 1935, **29**, 660, etc.; and others.

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1 Trevan, J. W., *Proc. Roy. Soc.*, 1927, **101**, 483.

2 Schønheyder, F., *Biochem. J.*, 1936, **30**, 890.

3 Almquist, H. J., and Stokstad, E. L. R., *J. Nutrition*, 1937, **14**, 235.

4 Almquist, H. J., Mecchi, E., and Klose, A. A., *Biochem. J.*, 1938, **32**, 1897.

5 Almquist, H. J., *J. Biol. Chem.*, 1936, **114**, 241.

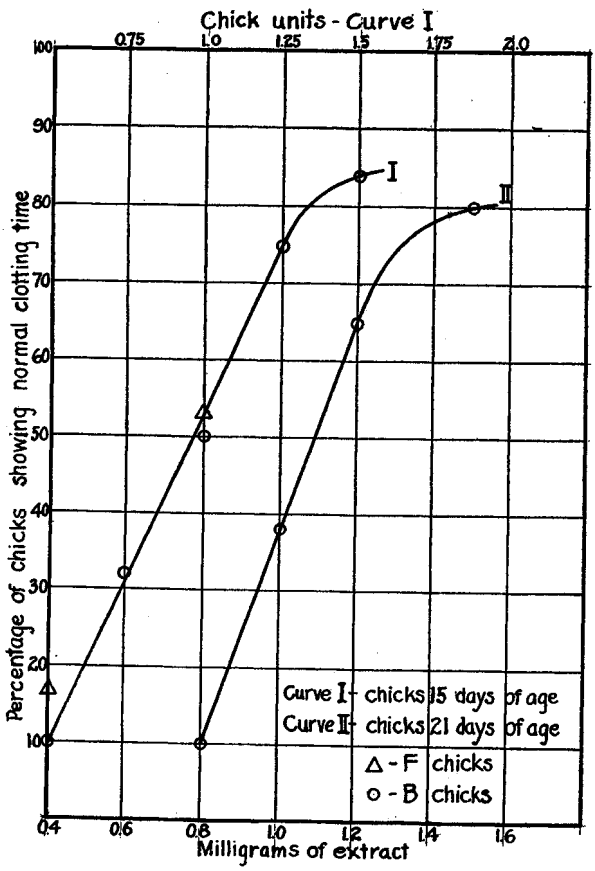

FIG. 1.

Using this diet it has been found that a severe deficiency can be produced within 2 weeks even in AAA grade chicks (B) obtained from a commercial hatchery. Chicks (F) from eggs obtained from hens which had been on a diet devoid of greens seemed to develop only a slightly greater degree of deficiency. (Fig. 1.)

Assay. The vitamin dissolved in 0.6 cc of sesame oil was given orally, 0.20 cc of solution being administered on each of 3 successive days.† The solution was administered from a tuberculin syringe through a tube (1x90 mm) which was inserted directly into the crop. On the fourth day approximately 0.5 cc of blood was drawn from the brachial vein into a small porcelain crucible, and the time for coagula-

† Perhaps the single administration used by Ansbacher (*Science*, 1938, **88**, 221) may prove to be more satisfactory than the divided administration employed in our work. Certainly, the shorter time required for assay is a point in its favor.

tion at room temperature determined. A clotting time of 10 minutes or less was arbitrarily considered normal.

In the preparation of the curves 200 B chicks 21 days of age, 250 B chicks and 100 F chicks 15 days of age were used. (This study differs from that previously reported by Thayer, *et al.*[6]) By a series of different quantities (for Curve I, 0.4, 0.6, 0.8, 1.0, and 1.2 mg) we obtained responses which expressed in terms of percentage were plotted against the dosage (Fig. 1). These points fall upon a curve similar to that found by Trevan in his fundamental studies of bioassay.

Chicks 15 days of age have been used in our later work for the following reasons: 1st, mortality is considerably less in attaining the age at which they are used (25% for the chicks 15 days of age and 50% for chicks 21 days of age); 2nd, food consumption is not as great; 3rd, the amount of care required by the chicks is reduced by the shorter period; and 4th, with the same caging facilities more assays can be conducted.

Our unit is that quantity of vitamin which produces a clotting time of 10 minutes or less in 50% of a group of ten or more chicks which has been fed for the 14 days immediately following receipt from the hatchery on a diet practically devoid of Vitamin K. The chart is so constructed that having the response in percentage the corresponding value in chick units is immediately apparent from the curve.

The accuracy of the method was checked by the administration of "unknowns" prepared from the extract which had been used in the preparation of the curves, groups of 10 chicks being used for each assay. In the assay of an unknown, it may be necessary to use several groups of chicks as it is necessary to find the dosage, the administration of which produces a response that falls on the curve.

TABLE I.
Accuracy of the Method.

Standard preparation administered as unknowns		Response	Found from standard curve		Error
mg	Chick units	% positive	mg	Chick units	%
0.65	0.8	50	0.80	1.0	+23
1.00	1.3	60	0.90	1.1	—10
0.95	1.2	50	0.80	1.0	—16
1.15	1.4	80	1.06	1.3	— 8
0.70	0.9	33*	0.58	0.7	—17

*1 chick died.

[6] Thayer, S. A., MacCorquodale, D. W., McKee, R. W., and Doisy, E. A., *J. Biol. Chem.*, 1938, **123**, CXX.

preferably near its midpoint. Examination of Table I shows that our method gives fairly good assays. However, a statistical study indicates that more accurate results can be obtained by using 20 or 30 chicks for each assay.

Our more recent experiences indicate that the degree of deficiency produced in 15 days in different shipments of chicks may show considerable variation. For this reason we determine the response of each batch of chicks to a standard preparation of Vitamin K. Over a period of the last 7 weeks the same dosage of the standard preparation was administered to 8 groups of approximately 10 chicks; the responses obtained were 40, 44, 50, 38, 50, 60, 70, and 50%. Moreover, we advise that before starting assays with a particular lot of chicks, that the clotting time of 10 or more should be determined to ascertain the degree of deficiency.

In order that other investigators may obtain an idea of the magnitude of our unit we find that one gram of the artificially dried alfalfa used in our work yields approximately 10 units of the vitamin. For these assays the alfalfa meal was exhaustively extracted with petroleum ether, the solvent distilled and the residue taken up in sesame oil.

In our early work in which the deficiency was less pronounced, the potency of our most highly purified concentrate was about 1000 units per milligram. In later work in which a more severe deficiency has been produced we find about 1000 units per milligram even though we are confident that the purity of the vitamin is definitely increased.

Summary. A curative method of assay of Vitamin K based on Trevan's principles of bioassay has been found to give satisfactory results.