through the testicle, with no evidence of diminution in virulence as tested by the cerebral route.

We have found that the virus multiplies in the testicle with facility and in 3 days reaches a titer of 10^8 M.I.D. per testicle. The titer is approximately the same as that reached in the brain after intracerebral inoculation. This high virus content of the testicle persists for at least 2 weeks, since dilutions of testicular tissue of 10^{-6} are still infectious for mice at the end of this time; titrations at intervals longer than this have not been done as yet.

Levaditi and Lépine,[3] who compared the effects of various routes of inoculation of the St. Louis encephalitis virus in mice state that an occasional animal succumbs to encephalitis following testicular inoculation, but the majority remain unaffected and become resistant to cerebral introduction of virus. Most of our animals showed no ill effects during our observation period of 3 weeks; the occasional deaths were preceded by listlessness and ruffling of fur, and at no time did we note the occurrence of definite cerebral symptoms. Bacteriologic cultures of brain material from these mice were sterile and cerebral passage to test mice did not result in encephalitis.

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*The Assay of Vitamins K_1 and K_2.

SIDNEY A. THAYER, R. W. MCKEE, S. B. BINKLEY, D. W. MACCORQUODALE AND EDWARD A. DOISY.

From the Laboratory of Biological Chemistry, St. Louis University School of Medicine, St. Louis.

To explore more thoroughly the potencies of Vitamins K_1 and K_2, we have assayed the two pure compounds by 3 different procedures and a modification of one of them. The methods are: 1. The procedure previously described by us.[1] 2. A procedure suggested by Ansbacher's[2] work but differing from the method[3] which he finally

[3] Levaditi, C., and Lépine, P., *Les ultravirus des maladies humaines*, 1938, Librairie Maloine, Paris, V. 1, 513.

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[1] Thayer, S. A., McKee, R. W., Binkley, S. B., MacCorquodale, D. W., and Doisy, E. A., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 478.

[2] Ansbacher, S., *Science*, 1938, **88**, 221.

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

adopted. 3. A procedure very similar to that used by Almquist[4] but differing in the details of the actual determination of clotting time. 4. A procedure differing from (3) only in that the vitamin dissolved in 0.20 cc of sesame oil was administered separately each day.

The basal diet used in all of our experiments is one described by Almquist. 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. Using this diet it has been found that a severe deficiency can be produced in AAA grade chicks within 2 weeks.

As previously defined our unit is that quantity of vitamin which produces a clotting time of 10 minutes or less in 50% of a group of 10 or more chicks which have been fed for 14 days immediately following receipt from the hatchery on a diet practically devoid of Vitamin K. Our experience indicates that the degree of deficiency of different lots of chicks varies considerably but that the main variation seems to be seasonal. During the late winter and early spring the deficiency is greatest. At the time we adopted 0.8 mg of our standard preparation as a unit 50% of the birds responded. At the present time the deficiency is more severe and consequently a larger dosage is required to produce a 50% response. Because of this variation in the degree of deficiency we have found it necessary to standardize our chicks with our standard extract if we are always to find approximately the same potency for the pure compounds.

As shown in our previous correspondence,[1] by means of the dosage-response curve the number of units in an unknown preparation can be determined by obtaining the percentage response of the chicks to a known amount of the preparation. A parallel assay of the standard is carried out during the same period with a group of comparable chicks. This procedure enables one to correct for any variation in deficiency of a given group of chicks.

Using the dosage-response curve and our data (No. 1) in Table I, 69% and 18% responses correspond respectively to 0.95 and 0.47 mg of our standard. Since 0.8 mg is by definition 1 unit and the response to 0.8 mg of this particular group of birds was 18%, 2 micrograms of Vitamin K_1 is equal to 0.95/0.47 or 2 units. One microgram is therefore equal to 1 unit and the potency of K_1 is 1000 units per milligram. The calculations for 2, 3 and 4 of Table I were carried out by the same procedure.

Since the speed of the 18-hour assay procedure would greatly

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

expedite our work, we have determined the dosage-response curve to our standard preparation. Although fewer chicks have been used in this work than were employed in the construction of the curve for the 3-day procedure it appears that the 50% response is given by about 70% of the dosage which was required to produce a 50% response in the 3-day method.† The curves are parallel but the curve for the 18-hour procedure lies to the left of the curve for the 3-day method. The data given in Table I under the 18-hour procedure were used according to the principles of the preceding paragraph in calculating our standard units per milligram for K_1 and K_2.

In view of the interesting work on Vitamin K conducted by Almquist and his collaborators it has seemed desirable to ascertain how his assays compare with ours. Chicks that had been maintained on the basal diet for 7 days after delivery from the hatchery were divided into test groups of 10 chicks (5 chicks to a cage). They were

TABLE I.

No.	Products tested	Amt of compound administered orally (micrograms)	No. of chicks (15 days of age)	Response (normal clotting time) % positive	Dosage equivalent to percentage response, mg	Our standard units per milligram
			3-Day Assay Method.			
1.	Vitamin K_1 (alfalfa)	2.0	16	69	.95	1000
	Standard prep.	800.0	16	18	.47	
2.	Vitamin K_1 (alfalfa)	1.5	9	55	.82	1000
	Standard prep.	800.0	8	25	.54	
3.	Vitamin K_2 (fish meal)	1.5	10	30	.59	660
	Standard prep.	800.0	10	30	.59	
4.	Vitamin K_2 (fish meal)	2.0	10	70	.96	770
	Standard prep.	800.0	9	33	.62	
			18-Hour Assay Method.			
5.	*a.* Vitamin K_1	0.75	8	37	.44	770
	b. "	1.00	8	50	.57	740
	Standard prep.	600.0	8	50	.57	
6.	Vitamin K_2	2.00	10	70	.77	500
	Standard prep.	800.0	10	70	.77	
7.	Vitamin K_2	2.00	20	70	.77	480
	Standard prep.	400.0	18	33	.40	

† Actually, with birds of the *same degree of deficiency* approximately twice as much vitamin is required for a 50% response by the 3-day procedure. However, this is not a factor to be considered in this discussion.

TABLE II.
Slightly Modified Almquist 7-day Curative Method.

No. of chicks	K_1 per kg of diet expressed in micrograms	Avg clotting time for each test group in minutes	No. of chicks	K_2 per kg of diet expressed in micrograms	Avg clotting time for each test group in minutes
9	40	10.1	9	80	11.2
9	80	5.8	8	160	6.4
8	160	4.6	10	240	3.2
9	160	4.6	8	320	3.0

Of 23 control chicks 3 had clotting times of 31, 32 and 45 minutes, and 20 clotting times of over 1½ hours. All of the controls except the one with clotting time of 31 minutes bled to death.

kept in a dimly lighted room and were fed on the basal diet supplemented with Vitamin K for 7 days. Groups of 10 negative controls were started on the basal diet at the same time, but usually some of these died before the experimental period, 7 days, had elapsed. We deviated from the Almquist procedure in that we followed our usual procedure in obtaining blood samples and determining the clotting time; however, the results are so consistent and clear-cut that it is not likely that any appreciable difference in results was introduced. The results are shown in Table II.

These data indicate that Vitamin K_1 is approximately twice as potent as K_2 and confirms our previous observations. During the 7-day test period 80 micrograms per kilo of diet for Vitamin K_1 and 160 micrograms per kilo for K_2 are adequate for restoring a normal clotting time. Since a chick ingests approximately 75 g of food during the 7-day period the requirement of Vitamin K_1 for each chick per day is approximately 0.9 micrograms or 1.8 micrograms of K_2.

In addition to the administration of the vitamins mixed with the food the vitamins dissolved in oil have been given daily to each chick during the 7-day test period. Under these conditions approximately 0.45 micrograms of K_1 or 0.9 micrograms of K_2 is the daily requirement for the chicks. By the use of this technic, it is certain that each chick receives a definite amount of the vitamin and moreover the possibility of loss of vitamin by decomposition is diminished.

Summary. 1. The potency of Vitamin K_1 is approximately 1000 of our units per milligram; K_2 approximately 660. 2. The 18-hour assay procedure gives satisfactory results. 3. In a slightly modified Almquist 7-day curative method, 80 micrograms per kilo of diet of K_1 and 160 micrograms per kilo of diet of K_2 are adequate.