TABLE III.
Minimum Effective Doses in 6- and 18-hour Tests.

Substance	6 hr	18 hr	Ratio
	γ	γ	
Vitamin K_1	15	1	15:1
Methylnaphthoquinone	½	¼	2:1
Alfalfa Concentrate	2	1	2:1

potency of 1 unit in about 30 γ are quite complex in composition and contain several chemical individuals with varying vitamin K potencies, as investigations by chromatographic analyses on calcium sulfate show. Some of these fractions are deep red, others are yellow, but our most potent concentrates were always nearly colorless. It should be noted that our process of isolation is different from that employed by other investigators and this may be one of the reasons why we were able to obtain concentrates which had such a high potency but did not give the typical color reaction for vitamin K_1.

11009

Nitrogen Retention on Casein Digests.

WARREN M. COX, JR., AND ARTHUR J. MUELLER.
From the Mead Johnson Laboratories, Evansville, Indiana.

Early work with digests of casein showed that nitrogen retention and growth in young dogs and rats might be promoted[1, 2] by digests prepared by the combined action of digestive juices. In these studies and in those of Hopkins[3] it was recognized that acid digests were incomplete mixtures and had to be supplemented by the addition of tryptophane. Not all workers have been able to confirm these original findings.[4] This may have been due to the quality of the hydrolysates employed. Enzymic hydrolysis, according to Abderhalden's procedure, involves the use of gastric, pancreatic and intestinal extracts, and digestion for a considerable period of time. Boiling hydrochloric or sulfuric acid of the concentration ordinarily used for acid hydrolysis may result in the formation of by-products (amines?) which, in the absence of adequate purification procedures, may mask the actual nutritive value of the digest. A purified casein digest prepared with

1 Abderhalden, E., and Oppler, B., *Z. physiol. Chem.*, 1907, **51**, 226; *J. C. S.*, 1907, ii A, 369.

2 Abderhalden, E., *Z. physiol. Chem.*, 1915, **96**, 1.

3 Hopkins, F. G., *J. Chem. Soc.*, 1916, **109**, 629.

4 McClendon, J. F., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 915.

pancreatic enzymes has been made in this laboratory and has been shown to be satisfactory for the growth of rats, and for the regeneration of serum protein in dogs, when assayed in comparison with the unhydrolyzed casein.[5] Acid digests have also been made and carefully purified. Both preparations, after drying, were light, impalpable powders, almost white. On dissolving they gave straw-colored solutions. The present report deals with the nitrogen balance obtained with both types of digest.

Experimental. Adult male rats were placed in metabolism cages adapted to separate urine from feces and to minimize spilling of the food. The rats were first placed on stock diet until they became accustomed to the cage and were then transferred to the nitrogen-low diet. The composition of the basal diet was:

Maltose and Dextrins*	57.0
Olive Oil	22.7
Starch (arrowroot)	12.4
Salts†	7.9

The various ingredients were mixed with water, heated to boiling, homogenized and dried in a pilot scale spray-drier. Analysis of the powder showed the following percentage composition: protein, 0.25; fat, 23.04; ash, 4.60; moisture, 0.81; carbohydrates (by difference), 71.30.

Necessary vitamins were supplied by one yeast tablet (.39 g, containing approximately 20 I.U. of B_1 and 20 Sherman units B_2) on which was placed one drop of Oleum Percomorphum daily. The total nitrogen intake from the basal diet and vitamin supplements was between 30 and 38 mg per day.

Feces and urine were collected daily and separately; food and water intake was carefully measured. Feces were prepared for analysis by grinding, and treatment with sulfuric acid according to the method of MacKay and Butler.[6] All nitrogen determinations were made in duplicate by micro-Kjeldahl.

Comparison of Casein and Enzymic Casein Digest. In Fig. 1, 68 daily nitrogen balances for 7 periods of observation are graphically presented. The fecal and urinary nitrogen excretions are indicated by cross-hatched and white areas on the lower half of the chart. The

[5] Mueller, A. J., Kemmerer, K. S., Cox, W. M., Jr., and Barnes, S., in press.

* Dextri-Maltose.

† Calcium gluconate, 4.0; Monobasic potassium phosphate, 1.9; Calcium hydroxide, 1.0; Potassium chloride, 0.5; Dibasic potassium phosphate, 0.5; Magnesium oxide, 0.05.

[6] Peters, J. P., and Van Slyke, D. D., *Quantitative Clinical Chemistry*, Williams and Wilkins, Baltimore, 1932.

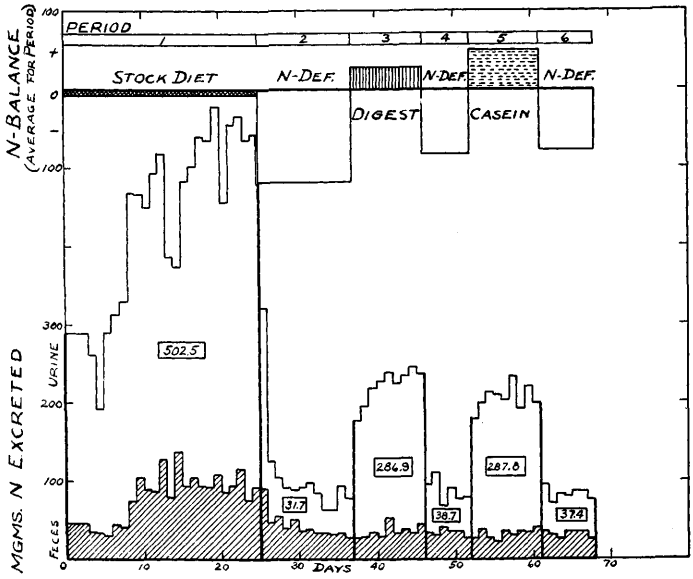

FIG. 1.

Daily nitrogen excretion and nitrogen balance of adult male rat receiving stock diet (Period 1) and a nitrogen-free basal diet (Periods 2, 4 and 6) supplemented with an enzymic digest of casein (Period 3) and casein (Period 5).

average nitrogen balances for the period are given graphically at the top and the nitrogen intake numerically in blocked squares. During Period 1, stock diet *ad libitum,* food intake was initially quite low, and even though large positive nitrogen balances were obtained after the rat had become accustomed to the surroundings, the average balance for the 25 balance periods was –8.7 mg/day. Large negative balances followed the substitution of the basal, nitrogen-low diet (Period 2) with prompt drop in both urinary and fecal excretion of nitrogen.‡

The enzymic digest of casein (92-Z) prepared as described elsewhere,[5] was mixed with a small amount of the basal diet and avidly consumed by the rat during Period 3. The nitrogen balance was positive (27 mg/day), and the urinary and fecal excretion remarkably constant throughout the period. In order to facilitate the comparison only 2 g of the digest (251 mg N) was fed, although the nitrogen intake on stock diet averaged about 500 mg N per day. The total nitrogen intake was, therefore, approximately half of the usual intake.

Acid-washed, vitamin-free casein was fed at the same nitrogen

‡ The nitrogen intake during the first period on the basal diet is lower than on later periods because for the first few days, until nitrogen deficiency was prominent, the yeast tablet was not all eaten.

intake level, and, as will be noted (Period 5), with slightly greater nitrogen retention (49 mg/day). The urinary excretion of nitrogen was approximately the same as when the digest was fed. The conclusion seems warranted that both casein and an enzymic digest of casein prepared as described, result in positive nitrogen retention.

Retention on Acid and Enzymic Casein Digests. Acid hydrolysis of protein results in the complete destruction of tryptophane, and the resulting mixture of amino acids is nutritionally inadequate. It was desirable, however, to determine if any of the nitrogen of such a preparation might be retained or whether feeding such a preparation would be equivalent to subsistence on a low-nitrogen diet. Evidence of decreased nitrogen loss and even of slight positive retention on nutritionally inadequate mixtures of amino acids has recently been presented by Nielsen and Corley.[7]

Fig. 2 shows the nitrogen retention on the enzymic digest (Period 2) and on the acid digest (89) supplemented with 0.2% tryptophane (Periods 4 and 6). Both supplements were greedily consumed by the animal. Following Period 4, and without an intervening period on the basal diet, the tryptophane was omitted. On the first day all the supplement was again consumed (with a resultant increase in

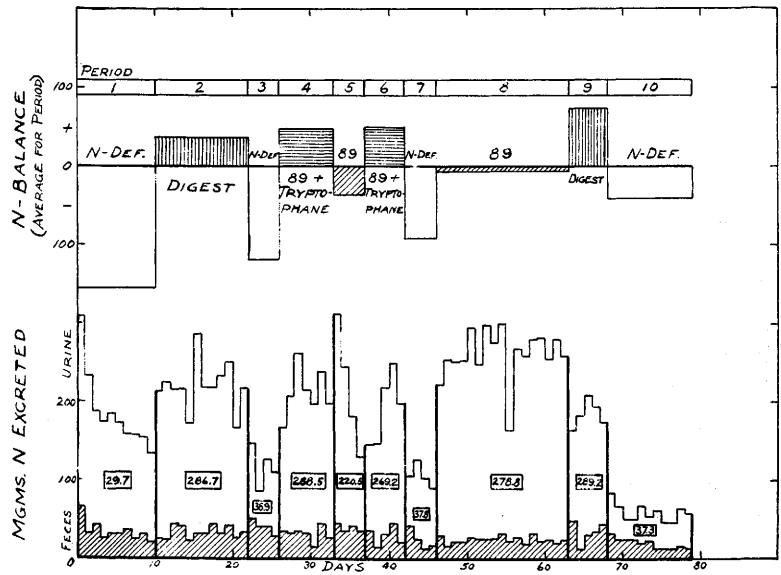

FIG. 2.

Daily nitrogen excretion and nitrogen balance of adult male rat receiving a nitrogen-free basal diet (Periods 1, 3, 7 and 10) supplemented with an enzymic digest of casein orally (Period 2), and by stomach tube (Period 9); with an acid digest of casein orally (Period 5) and by stomach tube (Period 8); and the latter plus tryptophane (Period 4 and 6).

[7] Nielsen, E. K., and Corley, R. C., *J. Physiol.*, 1939, **126**, 223.

urinary nitrogen), but on the subsequent 3 days progressively less was eaten until on the fourth day only 91 of the 250 mg nitrogen offered was ingested. This decrease in intake is indicated by the rapid drop in urinary excretion during Period 5.

Tryptophane was then added to the supplement and after 2 days of cautious approach (indicated here by low urinary excretion), it was again eaten greedily, with resultant positive balances. This ability of the animal to distinguish between nitrogen sources that are adequate and those that are inadequate has been frequently observed.[8] The daily urinary excretions, however, give an excellent picture of the rapidity with which an animal can adjust for changes in adequacy.

To get a true comparison, it was necessary to administer the acid digest by stomach tube (Period 8). Half of the total amount (2 g = 250 mg N) was given in the morning and half in the afternoon. Daily nitrogen balances were run for 17 days. As will be noted (Period 8), urinary excretion was quite constant (on the tenth day only half of the required amount was given) and there was a small average negative nitrogen balance for the period. The magnitude of the loss was considerably less than on the nitrogen-low basal diet alone, and would indicate that a considerable portion of the acid hydrolysate was utilized or stored by the body. During this 17-day period, weight declined from 356 to 339 g for an average loss of one gram per day, indicating that in spite of the approximate balance in nitrogen metabolism, the mixture was far from nutritionally adequate. While this is to be interpreted as a severe weight loss, it was not so severe as that recorded on the basal diet. There were 4 such periods (1, 3, 7, 10) and the daily weight loss for each respectively was 7.1, 3.7, 2.0, and 2.9 g. The animal thus fared better than if no form of nitrogen had been given. It might be assumed that the small amount of tryptophane from the vitamin supplement and possibly in the basal diet was the factor promoting a partial utilization of the incomplete acid hydrolysate.

This period was followed immediately by a period of 5 days during which an identical amount of nitrogen, in the form of the enzymic hydrolysate, was given by stomach tube (Period 9). Several things of interest are to be noted: weight was stationary at 337 g, the animal went into large positive nitrogen balance, and the urinary excretion of nitrogen declined sharply. The picture is entirely different from that obtained with the acid hydrolysate, and suggests that all essential amino acids were present in the digest prepared by enzymic action.

Summary. 1. Continuous daily nitrogen balances covering 140

[8] Rose, W. C., *Physiol. Rev.*, 1938, **18**, 109.

days have been made on rats restricted to a nitrogen-low basal diet, supplemented for various periods with casein, and an acid or an enzymic digest of casein. 2. The enzymic digest fed in comparison with casein, resulted in nitrogen retention of approximately the same magnitude as the unhydrolyzed protein. 3. The acid digest, supplemented with 0.2% tryptophane likewise gave positive nitrogen balances, but without the addition of the essential tryptophane, was refused by the animals. 4. When both the unsupplemented acid digest, and the enzymic digest were administered by stomach tube, the latter was well retained, but the former gave a slight negative balance with some indication that it was partially effective in supplying animal requirement for nitrogen.

11010

Quantitative Biological Assay of Vitamin K and Its Application to Several Quinone Compounds.*

FLEMINTINE PEIRCE DANN. (Introduced by Carl Nielsen.)

From the Abbott Laboratories, North Chicago, Illinois.

The Biological Assay Technic. When this work was started in 1937, the 2 methods used for the biological assay of the antihemorrhagic factor were the curative technic of Schønheyder[1] and the preventive method of Almquist and Stokstad.[2] The Schønheyder method which depends on a curative effect is theoretically more accurate than a preventive one. In the application of the method, the long and involved technic of taking blood samples and determining the blood clotting time is a definite disadvantage. Later, Dam and Glavind[3] revised the Schønheyder method without simplifying these steps.

In the meantime, some work[4] had been done in our laboratory on the Almquist and Stokstad preventive method. Finally, a method for the biological assay of the antihemorrhagic factor was developed,

* The author is indebted to Dr. H. Dam for his standard spinach tablets, to Dr. R. J. Anderson for the phthiocol compound, to Dr. Byron Riegel for a sample of the natural vitamin K_1, to Dr. E. A. Doisy for a specimen of his natural vitamin K_1, and to Dr. L. F. Fieser for a sample of the synthetic vitamin K_1.

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

2 Almquist, H. J., and Stokstad, E. L. R., *J. Nutri.*, 1937, **14**, 235.

3 Dam, H., and Glavind, J., *Biochem. J.*, 1938, **32**, 1018.

4 Dann, F. P., *Am. J. Physiol.*, 1938, **123**, 48.