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Isolation of Vitamin K_2 from Cultures of a Spore-Forming Soil Bacillus.

M. TISHLER AND W. L. SAMPSON. (Introduced by H. Molitor).

From the Merck Research Laboratories and the Merck Institute for Therapeutic Research, Rahway, N. J.

During the course of an investigation of the bactericidal substances obtained from a spore-forming soil bacillus, *Bacillus brevis,* described by Dubos and Cattaneo,[1] we observed that ether extracts of the autolyzed bacilli gave a Dam-Karrer color reaction with alkali, characteristic of vitamin K.[2] When the oily residue obtained from the ether extracts was tested for antihemorrhagic activity in chicks[3] it was found to be fully effective at 100γ.

The K principle present in this residue was readily isolated by the method of Fieser for the isolation of vitamin K_1 from alfalfa concentrates.[4] Thus, according to this procedure 8 g of the above residue was suspended in 75 cc methanol to which was added 1 g of sodium hydrosulfite dissolved in 3 cc water. After shaking for 18 hours, the dihydrovitamin was taken into petroleum ether, washed with 2% aqueous potassium hydroxide containing sodium hydrosulfite until the washings were colorless, extracted with Claisen's alkali containing hydrosulfite; and recovered from the bright yellow liquor by dilution with water and extraction with petroleum ether. As the reduced form of this K factor is somewhat more soluble than dihydro-vitamin K_1,[4] the petroleum ether solution (2 cc) was chilled to −20°C, and the waxy solid which deposited was separated by centrifugation. After washing several times with petroleum ether by centrifugation at −20°C, the white, waxy hydroquinone dissolved in anhydrous ether was oxidized with silver oxide in the presence of anhydrous magnesium sulfate. On concentrating the ether solution, a bright yellow oil was obtained (80 mg) which crystallized after the addition of a few drops of cold acetone. By recrystallization from a mixture of chloroform and methanol the product was obtained as microscopic, yellow platelets which was identified as vitamin K_2 by a comparison of its properties with those already reported.[5] The product melted at 52.5 - 53.5°C (Found: C, 84.45; H, 9.99), and on reductive acetylation the dihydrodiacetate melting at 57 - 58°C was obtained (Found: C, 80.94; H, 9.86). In hexane, the ultraviolet absorption indicates sharp maxima at 243, 249 ($E^{1\%}_{1\,cm} = 520$), 260, 269 and a broad and less intense band at 310 to 340 with a maximum very close to 325. A comparative chick assay of vitamin K_2 with vitamin K_1 is shown in Table I. These results are in excellent agreement with those previously reported.[6]

Since ether extracts of the nutrient used for growing the organism (Bacto-Tryptone, Difco) were found to have some K activity, it was of interest to demonstrate whether the K_2 present in the incubated culture is actually

TABLE I.

Substance	Dose, μg	No. of chicks	Clotting time below 10 min.
K_1	1	9	8
K_2	1	9	6
	1.5	9	8
	2.0	9	9
Control	0	9	all 60 min.

[1] Dubos and Cattaneo, *J. Exp. Med.*, 1939, **70**, 249.

[2] Dam, Geiger, Glavind, P. Karrer, W. Karrer, Rothchild, and Solomon, *Helv.*, 1939, **22**, 310.

[3] Tishler and Sampson, *J.A.C.S.*, 1939, **61**, 2563.

[4] Fieser, *J.A.C.S.*, 1939, **61**, 2561.

[5] McKee, Binkley, MacCorquodale, Thayer, and Doisy, *J.A.C.S.*, 1939, **61**, 1295; Binkley, MacCorquodale, Cheney, Thayer, McKee, and Doisy, *J.A.C.S.*, 1939, **61**, 1612; McKee, Binkley, Thayer, MacCorquodale, and Doisy, *J. Biol. Chem.*, 1939, **131**, 327.

[6] Thayer, McKee, Binkley, MacCorquodale, Doisy, PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 194; Dam, Glavind, and Karrer, *Helv.*, 1940, **23**, 225.

produced by the organisms. Chick assays and quantitative analyses by the method of Trenner and Bacher[7] developed for vitamin K_1 proved that the yield of vitamin K_2 from the autolyzed bacilli is not significantly altered when the nutrient is previously extracted with ether. Furthermore biological assays on the nutrient itself indicated that not more than 10% of the vitamin present in the final culture can be introduced from this source.

The synthesis of a substance with K activity during putrefaction of fish meal and rice bran was first pointed out by Almquist and Stokstad.[8] It was found later that species of bacteria grown on the usual media also produced an antihemorrhagic factor.[9] No attempt, however, was made by these investigators to isolate and characterize the active principle. The well-known isolation of K_2 from putrefied fish meal[5] and this report of its isolation from the autolyzed cellular material of a spore-forming soil bacillus suggest that vitamin K_2 is a bacterial metabolite. In this connection the observation of Woolley and McCarter[10] that antihemorrhagic compounds as vitamin K concentrate, 2-methyl-1, 4-naphthoquinone, and phthiocol stimulates the growth of Johne's bacillus (*Mycobacterium paratuberculosis*) is noteworthy.

Summary A substance has been isolated from the autolyzed cellular material of the spore forming soil organism *Bacillus brevis* which, by both chemical and biological examination, is identical with vitamin K_2.

7 Trenner and Bacher, *J. Biol. Chem.*, 1941, **137**, 745.

8 Almquist and Stokstad, *J. Nutrition*, 1936, **12**, 329.

9 Almquist, Pentler, and Meech, PROC. SOC. EXP. BIOL. AND MED. 1938, **38**, 336.

10 Woolley and McCarter, PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 357.

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Effect of Amino Acid Deficiency on Rat Liver Before and After Partial Hepatectomy.*

DAVID RAPPORT, ATTILIO CANZANELLI, AND RUTH GUILD.
From the Physiology Department, Tufts College Medical School, Boston, Mass.

Since the remaining portion of rat liver after partial hepatectomy regenerates to normal size within 10 to 14 days it seemed of interest to see what effect a dietary deficiency of some essential amino acids might have on the formation of nucleic acids during this period of extremely rapid growth.

Partial hepatectomy was performed on rats that had been fed diets whose protein content was exclusively casein, zein, or gelatin. The livers at the time of hepatectomy and 4 days later were analyzed for ribonucleic acid, desoxyribonucleic acid, tryptophane, percentage regeneration, total nitrogen and total solids. Values for ribonucleic acid (P.N.A.) and desoxyribonucleic acid (D.N.A.) were obtained by the method of Schmidt and Thannhauser.[1] The determination of tryptophane was made by the procedure of Horn and Jones.[2] The percentage regeneration of the liver after partial hepatectomy is the increase in weight of the remnant as percentage of the amount of tissue removed. Since, on

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1 Schmidt, Gerhardt, and Thannhauser, S. J., *J.B.C.*, 1945, **161**, 83.

2 Horn, M. J., and Jones, D. B., *J.B.C.*, 1945, **157**, 153.