Production of Dietary Vit. K Deficiency in the Rat. (24982)

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It has been frequently stated that the rat, among other mammalian species, has no dietary requirement for Vit. K and that intestinal bacterial synthesis of this vitamin is adequate for normal blood coagulation(1). To produce Vit. K deficiency in the rat, sulfa drugs are usually administered to depress intestinal microbial synthesis(2), or flow of bile into the small intestine is arrested to diminish absorption of Vit. K(3). Prevention of coprophagy has recently been reported to result in Vit. K deficiency in rats fed a Vit. K-deficient diet(4), indicating that Vit. K microbially synthesized in the intestinal tract was not directly absorbed but obtained by recycling of the feces. Rats fed gamma-irradiated beef died from non-specific hemorrhages which appeared to be due to a severe but uncomplicated Vit. K deficiency(5). This diet was very low in Vit. K and produced feces which were not acceptable to the rat. The purpose of this report is to describe a purified diet which when fed to weanling male albino rats produced a severe dietary Vit. K deficiency, as indicated by hypoprothrombinemia and lethal hemorrhages, without recourse to drugs, surgery or coprophagy prevention.

Methods. Weanling male albino rats of Sprague-Dawley strain were housed individually in wire-bottomed cages in temperature-controlled laboratory. Feed and water were provided *ad lib.* Composition of the diet is given in Table I. Coprophagy was prevented according to the method of Barnes *et al.*(6). The percent coprophagy refers to the proportion of total fecal dry matter consumed by the rat. Whole plasma prothrombin times were determined on 10th day of experiment according to the method of Quick(7). Hemorrhagic deaths were characterized by one or more of the following symptoms at autopsy: testicular hemorrhage, subcutaneous bleeding, hemothorax, and nasal bleeding. Oral Vit. K supplementation was carried out by administering water solution of tetra sodium salt of 2-methyl-1, 4-naphthoquinone diphosphoric acid ester by a calibrated eye dropper, to each rat at time of feeding. There were 6 rats in each group and the experiment lasted 10 days.

Results. Table II gives results obtained. It can be seen that rats consuming the diet described in Table I had abnormally long plasma prothrombin times and suffered from lethal hemorrhage. When coprophagy was prevented, the condition was worsened considerably. In either case, 5 μg of Vit. K_3/rat daily completely protected the rats from both hypoprothrombinemia and hemorrhage. The rats grew well in all cases. Gain in body weight was about 7.5 g/rat/day for first 7 days of experiment. Coprophagy on this diet was about 30 to 40%, about half that observed when casein was used in place of the purified soy protein (Drackett assay protein).

Discussion. The rat's physiological requirement for Vit. K can be satisfied by dietary Vit. K and/or by intestinal microbial synthesis. In large part, the latter source depends on the extent of coprophagy practiced by the rat, since very little, if any, of microbial Vit. K was available when coprophagy was prevented, as shown by development of a severe deficiency in a very short time when coprophagy was prevented. The diet described in

TABLE I. Diet Used to Produce Vitamin K Deficiency in the Rat.

Ingredient	%
Sucrose	66.5
Purified soy protein (Drackett assay protein)	20.0
DL-methionine	.5
Vitaminized cerelose*	5.0
Salts mixture No. 446†	4.0
Wheat-germ oil	.5
Cod-liver oil	1.5
Glycerol	2.0
	100.0

* Supplied the following in mg/kg diet: thiamine hydrochloride, 10; riboflavin, 10; calcium pantothenate, 50; pyridoxine HCl, 5; nicotinic acid, 20; folic acid, 1; vit. B_{12}, 0.10; biotin, 0.10; choline chloride, 1000.

† Mameesh *et al.*(8).

TABLE II. Effect of Coprophagy Prevention and Menadione Supplementation on Hemorrhagic Syndrome and Hypoprothrombinemia of Vit. K Deficiency in the Rat (10 Days).

Diet	Oral supplement/rat/day	No. of hemorrhagic deaths	Plasma prothrombin time, sec. (mean and spread)	Coprophagy, %
Soy protein		1	32 (14- 66)	38
Idem	5 μg vit. K_3	0	14 (13- 18)	29
" , coprophagy prevented		4	92 (63-120)	
Idem	*Idem*	0	15 (14- 16)	

Table I, besides being very low in Vit. K, also appears to lead to low fecal consumption by the rat in comparison to usual casein-synthetic diet and therefore produces a severe Vit. K deficiency in 10 days. 5 μg of menadione/rat/day were adequate for maintenance of normal plasma prothrombin time and completely protected the rats from hemorrhage.

Summary. A diet is described which produces a severe Vit. K deficiency in the rat in less than 10 days. The deficiency was manifested by prolonged plasma prothrombin times and hemorrhages. Daily menadione supplementation protected rats from the deficiency. Coprophagy appeared to be the means by which Vit. K synthesized by the intestinal microflora becomes available to the rat.

1. Owen, Charles A., Jr., in *The Vitamins* (W. H. Sebrell and R. S. Harris, editors), Academic Press, N. Y., 1954, vII, p422.

2. Black, S., Overman, R. S., Elvehjem, C. A., Link, K. P., *J. Biol. Chem.*, 1942, v145, 137.

3. Greaves, J. D., *Am. J. Physiol.*, 1939, v125, 429.

4. Barnes, R. H., Fiala, G., *Fed. Proc.*, 1958, v17, 470.

5. Metta, V. C., Mameesh, M. S., Johnson, B. Connor, *J. Nutrition*, 1959, (in press).

6. Barnes, R. H., Fiala, G., McGehee, B., Brown, A., *ibid.*, 1957, v63, 489.

7. Quick, A. J., *Am. J. Clin. Path.*, 1940, v10, 222.

8. Mameesh, M. S., Johnson, B. Connor, *J. Nutrition*, 1958, v65, 161.

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Convulsant Effects of Mikedimide (3,3, Methylethyl Glutarimide) in Monkeys.* (24983)

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Mikedimide‡ (3,3, methylethyl glutarimide) has been recently introduced as a central analeptic with strong antagonism to barbiturates (1). Similarities in some of its actions in humans to those of pentamethylenetetrazole (Metrazol) (2-4) led to these investigations of its effects on a) normal, b) epileptic, and c) brain-operated non-epileptic monkeys.

Method. Tests were performed on 19 *Macaca mulatta* weighing 3-5 kg. In the first group of 11 monkeys, 5 had been made chronically epileptic by application of alumina cream to cerebral cortex (5) and 6 were unoperated normal controls. Mikedimide (0.5%) was injected intravenously "rapidly," *i.e.*, within 1-3 minutes at 1-4 ml/min, at intervals of 12 to 14 days. Clinical observations and electroencephalographic recordings were made for at least 30 minutes following injection; when clinical convulsive seizures occurred, sodium phenobarbital was promptly administered intravenously. Threshold convulsant doses were considered to be the least quantity which induced clinical convulsions. Thresholds for clinical convulsions to intramuscular Metrazol were also determined. In a second

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