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Metabolism of Vitamin K_1 (Phylloquinone) in Man

The K group of vitamins comprises two major chemical forms, both fat-soluble and widely distributed in nature: (1) Vitamin K_1, now known as phylloquinone, which is produced exclusively by plants. (2) Vitamin K_2, of which there is a series of compounds termed collectively the menaquinones, and produced only by microorganisms (Pennock 1966).

Both forms of the vitamin consist of a naphthoquinone ring with a methyl group in the 2 position of the ring, and a side-chain attached to the ring in the 3 position. Vitamin K_1 has a phytyl side-chain made up of four isoprene units linked together (Fig 1). The side-chain in the K_2 vitamins is of variable length and gives rise to a number of compounds known as the MK series, the number of isoprene units in the side-chain varying from 1–13 although only 4–13 are found in nature (Fig 1). Also shown in Fig 1 is menadione, a synthetic compound with relatively little biological activity.

The only established function of vitamin K_1 and menaquinones in man and other mammals is in the synthesis by the liver of four plasma coagulation factors, i.e. factor II (prothrombin) and factors VII, IX and X. The K vitamins bring about the carboxylation of the glutamic acid residues at the NH_2-terminal end of the polypeptide chains of these clotting factors, which thereby acquire the property of binding calcium ions and thus become biologically active in the coagulation mechanism (Stenflo 1974); but the biochemical mechanism by which the K vitamins bring about these molecular changes is unknown.

Our studies have been concerned with the metabolism of tritium-labelled vitamin K_1, and in this paper we review our findings and consider some of the outstanding problems.

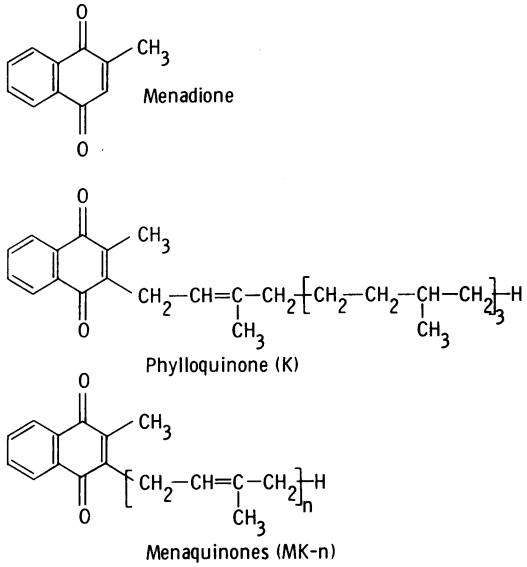

Fig 1 *Structural formulæ of menadione, phylloquinone (vitamin K_1) and menaquinones (vitamin K_2)*

Absorption of an Oral Dose of Vitamin K

Oral doses of 1 mg vitamin K_1 were detected in the plasma 30 min after ingestion, the levels then rising sharply to reach a peak at 2–4 hours (Shearer *et al.* 1970). These observations suggest that vitamin K_1 is absorbed mainly in the upper part of the small bowel. It is likely that vitamin K_1 is absorbed largely unchanged from the gut, since chromatographic analysis of plasma at the time of peak absorption showed that over 80% of the radioactivity was present as vitamin K_1 and associated mainly with large molecular size material, probably chylomicrons and low density lipoproteins.

The absorbed vitamin K_1 was cleared from the plasma relatively fast initially and then more slowly, the plasma level at 24 hr having fallen to 10–20% of the peak level (Shearer *et al.* 1970). Since the liver is the site of synthesis of the vitamin K dependent coagulation factor it is likely that most of the absorbed vitamin K_1 is removed from the plasma by the hepatic cells, but the precise tissue distribution in man is not known. The route of absorption of vitamin K_1 from the gut to the blood appears to be via the intestinal lymphatics (Blomstrand & Forsgren 1968).

A small amount of water-soluble radioactive material was found in the plasma, reaching a peak

at 2 to 4 hr after ingestion of the dose of vitamin K_1 and persisting at that level for about 24 hr, the level then falling over the next 72 hr (Shearer *et al.* 1970). It is likely that most of this water-soluble material is derived from the metabolism of vitamin K_1, largely in the liver, and its rapid appearance suggests that this metabolism commences rapidly and continues for some days. It is interesting that the plasma half-life of the circulating vitamin K dependent coagulation factors varies from a few hours for factor VII to about three days for prothrombin, with intermediate values for factors IX and X. This reflects their different rates of synthesis and could account for the rapid production of metabolites of vitamin K_1 and their presence over several days.

The amount of the oral dose of radioactive vitamin K_1 that was absorbed can be calculated from the amount of radioactivity recovered in the fæces (Shearer *et al.* 1970). About 20% of the ingested dose of vitamin K_1 was recovered as unchanged vitamin K_1 in the fæces, although the total excretion of lipid-soluble radioactivity was just over 50%. The difference between the total radioactivity and that of unchanged vitamin K_1 is due to the presence of vitamin K_1 metabolites believed to be derived from biliary excretion of vitamin K_1 that had been absorbed and then metabolized in the liver (Shearer *et al.* 1972). Because these vitamin K_1 metabolites account for most of the radioactivity excreted in the fæces, they complicate the assessment of the amount of an oral dose of vitamin K_1 that has been absorbed. Thus the true absorption is more accurately reflected by the difference between the administered dose and the amount of unchanged vitamin K_1 recovered in the fæces: on this basis about 80% of an oral dose is absorbed, suggesting an efficient mechanism for vitamin K_1 absorption.

Patients with cœliac disease and those with pancreatic insufficiency absorbed substantially less vitamin K_1 than normal subjects, and patients with complete obstruction of the common bile duct showed virtually no absorption (Shearer *et al.* 1974). In these patients the fæcal recovery of unchanged vitamin K_1 was higher than normal and that of altered vitamin K_1 lower than normal, reflecting the degree of malabsorption; treatment with a gluten-free diet and with 'cotazym' improved absorption in patients with cœliac disease and pancreatic insufficiency respectively (Shearer *et al.* 1974). Although absorption was reduced in patients with untreated cœliac disease or pancreatic insufficiency, significant amounts were absorbed, probably enough to supply the minimum daily requirements of vitamin K_1 since hypoprothrombinæmia of clinical significance is relatively rare in such patients. In contrast, patients with complete obstruction of the common bile duct usually develop a significant deficiency of the vitamin K dependent coagulation factors, reflecting the more severe reduction in the absorption of vitamin K_1.

Metabolism of an Intravenous Dose of Vitamin K_1

In normal subjects an intravenous dose of vitamin K_1 was cleared rapidly from the plasma, the clearance curves during the first six hours consisting of two exponential functions, the first with a half-life of about 22 min and the second with a half-life of about 135 min, representing a clearance of 90% of the injected dose in two hours and 99% in eight hours (Shearer *et al.* 1972).

About 36% of the injected radioactivity was recovered in the fæces after five days (most of this in the first 48 hr) while about 22% was recovered in the urine after three days (mostly in the first 24 hr). The greater part of the fæcal radioactivity was lipid soluble, and was attributable to altered vitamin K_1 as shown by thin-layer chromatography. Thus the major route of excretion of vitamin K_1 metabolites appears to be the biliary system.

In patients undergoing duodenal intubation, radioactivity was detected in the duodenal juice within 30 min after injection of the labelled vitamin K_1, suggesting that it was rapidly metabolized in the liver and excreted in the bile. Most of the radioactivity in duodenal juice and bile was associated with water-soluble material, in contrast with that in the fæces which was lipid soluble. It is likely that the material appearing in the fæces after the intravenous injection of vitamin K_1 is derived from biliary excretion of water-soluble conjugated metabolites, which become deconjugated and therefore lipid soluble during passage through the bowel.

The excretion of radioactivity in the urine approximated an exponential function, suggesting that the metabolism of vitamin K_1 proceeded at a constant fractional rate (Shearer *et al.* 1972).

Urinary Metabolites of Vitamin K_1

Two major acidic aglycone fragments were identified in the urine after oral or intravenous doses of vitamin K_1 (Shearer & Barkhan 1973). These metabolites retain the 1:4 naphthoquinone nucleus of vitamin K_1 but the side-chain is shortened to 5 carbon units in one fragment and to 7 carbon units in the other. Both metabolites were conjugated with glucuronic acid which rendered them water soluble.

Effect of Warfarin on Vitamin K_1 Metabolism

An oral therapeutic dose of warfarin produces a profound change in the plasma kinetics of an intravenous dose of tritiated vitamin K_1 (Shearer *et al.* 1973) and in its urinary metabolites (Shearer

et al. 1974). It was found that warfarin produced an apparent delay in the clearance from the plasma of the injected radioactivity and that this was due to the rapid accumulation in the plasma of phylloquinone oxide, but the rate of clearance of the injected vitamin K_1 (phylloquinone) was not different from that in untreated subjects. Furthermore, the excretion of radioactive metabolites in the urine was increased about twofold.

From their work in rats, Bell & Matschiner (1972) have postulated that vitamin K_1 in the normal course of its metabolism is first converted to the oxide, which must then be reduced back to vitamin K_1 (by a reductase) before it can exert its biochemical effect in the synthesis of the vitamin K dependent plasma coagulation factors, and they attribute the anticoagulant effect of warfarin to the inhibition of the conversion of vitamin K_1 oxide to vitamin K_1. This suggestion would explain our observation of the accumulation of vitamin K_1 oxide in the plasma in warfarinized subjects injected with vitamin K_1.

Sources of the K Vitamins for Man

It is widely believed that the vitamin K needs of man are supplied by the intestinal bacteria, but this has not been decisively established. Since the K vitamins are present in a wide variety of foods, both vegetable and animal, it is possible that man's main or only source of these vitamins is the diet, and since they are ubiquitous in nature and required in relatively small amount, a significant nutritional deficiency would be rarely encountered and very difficult to produce in normal humans. While the intestinal bacteria may produce menaquinones (vitamins K_2) there is no convincing evidence that these compounds are available for absorption in man. Vitamin K_1 has been instilled into the colon but no evidence of absorption was obtained (Udall 1965). Rats obtain menaquinones from their intestinal bacteria by coprophagy and therefore do not develop hypoprothrombinæmia when fed a vitamin K-free diet: however, the combination of dietary deprivation of vitamin K with prevention of coprophagy leads to hypoprothrombinæmia (Barnes & Fiala 1959) indicating that the vitamin K of the gut flora is not available *in situ* but, like that from the food or fæces, must traverse the oral route for digestion and absorption. Whether broad-spectrum antibiotics can produce vitamin K deficiency in man by suppressing bacterial growth in the bowel is therefore questionable and remains to be proved.

Daily Requirement of Vitamin K_1 in Man

The minimal daily requirement of vitamin K_1 to maintain a normal prothrombin time has been estimated to be in the range of 0.03–1.5 μg/kg (Frick *et al.* 1967). We have had the opportunity to study a patient with hypoprothrombinæmia and complete obstruction of the common bile duct (due to carcinoma of the pancreas) and to observe the effects of small intravenous doses of vitamin K_1 (Konakion) on the prothrombin time (PT) and the activated partial thromboplastin time (PTT). The patient weighed 80 kg and single intravenous injections of 40 μg, 10 μg and 2.5 μg were used in sequence. As shown in Fig 2, complete correction of the PT and PTT, maintained for 24 hr, was achieved with a single dose of 40 μg (about 0.50 μg/kg); a single dose of 10 μg (about 0.12 μg/kg) produced almost complete correction of the PT and complete correction of the PTT but maintained it for less than 24 hr; and, finally, an incomplete but substantial correction of both PT and PTT was obtained with three consecutive daily doses of 2.5 μg (about 0.03 μg/kg). These doses of vitamin K_1 are fairly near the range of the daily requirement proposed by Frick *et al.* (1967) in their study of comatose patients with cerebrovascular disease maintained on intravenous nutriments.

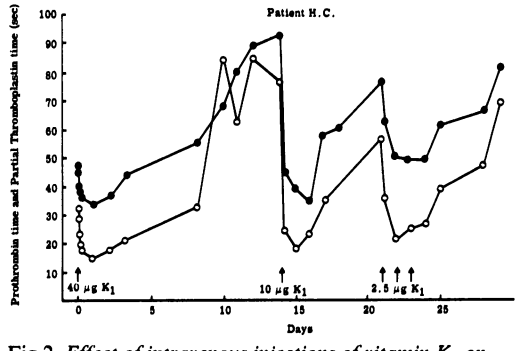

Fig 2 *Effect of intravenous injections of vitamin K_1 on the prothrombin time (normal range 12–14 s) and activated partial thromboplastin time (normal range 30 – 40 S) in a patient with complete obstruction of the common bile duct.* o–o, *prothrombin time.* •–•, *partial thromboplastin time*

While more patients with obstructive jaundice will need to be studied it is possible that the minimal daily requirement for vitamin K_1 in man may be in the range of 0.1 – 0.5 μg/kg. However, the diet of man will supply both vitamin K_1 and menaquinones (K_2) and the minimal daily requirement for the latter is not known. The biological activity of the menaquinones may be greater than that of vitamin K_1 and thus the minimum daily requirement for menaquinones may be lower than that suggested for vitamin K_1. Of course, a mixed diet would supply both K_1 and K_2, and the extent to which the daily requirement of the K vitamins for the synthesis of the K-dependent coagulation factors is met would depend on the relative amounts of these vitamins in the diet and therefore on the composition of the diet. The amount of

vitamin K in individual foodstuffs is largely unknown, and therefore the daily dietary intake remains to be established.

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