Relation of Vitamin E to Intestinal Flora and the Intestinal Absorption of Tocopherol

RECENTLY, J. J. Pindborg[1] reported that bacterial synthesis of vitamin E takes place in the digestive tract of the rat; and he might have cited experiments by Daft *et al.*[2] as presumptive confirmation. In both laboratories, rats which were subjected to sulpha drug-feeding to inhibit the activity of intestinal flora developed symptoms characteristic of vitamin E deficiency—muscle degeneration in Daft's laboratory and incisor teeth abnormalities and depigmentation in Pindborg's. If, however, extra vitamin E were administered, the deficiency syndromes did not develop. Pindborg suggested that intestinal micro-organisms had been synthesizing tocopherol, in amounts sufficient to meet the animals' needs, prior to the addition of sulpha drugs to the diet; afterwards, synthetic activity of the intestinal flora was diminished by the sulpha compounds, insufficient tocopherol was present in the intestinal tract, and vitamin E deficiency developed unless extra quantities of vitamin E were given as supplements.

We prefer a simpler explanation. Perhaps sulpha drugs, at a 1 per cent level in the diet, merely increase the vitamin E requirement of rats, as do many other dietary and environmental factors[3–5].

Pindborg also cited the positive results of a vitamin E bioassay of fæces from normal rats maintained on a good stock ration as definite direct evidence of vitamin E synthesis in the intestinal tract.

Again we prefer the simple explanation that any vitamin E present in fæces merely represents dietary tocopherols which have passed through the gastro-intestinal tract unabsorbed. Cuthbertson and associates[6], Engel and Heins[7], and Hines and Mattill[8] have already reported that tocopherol-like materials, measured by chemical and spectrographic procedures, are present in the fæces of rats receiving diets rich in vitamin E and are absent in excreta of animals maintained on vitamin E-low diets. We have confirmed these findings in our Laboratories and, in addition, have obtained the following relevant results.

Fæcal excretion of tocopherol-like reducing substances is proportional to the amount of tocopherols fed within wide limits (1–100 mgm. of tocopherols daily). Age of the animal is apparently not a factor in determining the extent of intestinal wastage of large doses of vitamin E, because experiments have been conducted on weanling rats, young fully grown adult rats, and senile rats, with almost identical results. The type of α-tocopherol fed, whether synthetic racemic or natural *d*-form, is apparently immaterial, while adjuvants such as bile salts and lecithin fed with the vitamin E are effective in increasing absorption of vitamin E.

Humans are similar to rats in their inability to absorb completely orally administered tocopherols. Two adult males were studied, and fæcal excretion of tocopherols was determined during metabolic periods of nine to sixteen days, with and without vitamin E supplementation.

Vitamin E-deficient rats waste somewhat less supplementary vitamin E than do normal adequately nourished rats, presumably because their tissues are avid for tocopherol, resulting in more efficient intestinal absorption. The tocopherol-like substances excreted during supplementation with vitamin E have physiological potency. They are, in part at least, unchanged tocopherols, because vitamin E bioassays of lipid extracts of fæces from both supplemented rats and humans have been positive.

α-Tocopherol labelled with radioactive carbon has been fed to rats and traced through the body and the gastro-intestinal tract. The results will be reported in detail later; but preliminary findings indicate that some actual labelled-tocopherol, ingested in doses of physiological size (1 mgm. per day), is excreted in the fæces.

We conclude from these findings that the explanation for vitamin E activity in fæces is due, not to bacterial synthesis in the intestinal tract, but to failure of complete intestinal absorption of dietary or supplementary tocopherols.

PHILIP L. HARRIS

Research Laboratories,
Distillation Products, Inc.,
Rochester 13, New York.
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Histochemical Study of Fat Deposits in Chronic Intoxication of the Dog by γ-Hexachlorocyclohexane

CHRONIC intoxication of dogs by the γ-isomer of hexachlorocyclohexane was induced by repeated intramuscular injections of 10–30 mgm. of this substance in 10 per cent oily solution per kgm body-weight, until a total dose of 130–475 mgm./kgm. was reached. The dogs died or were killed seven to forty-four days after the first injection. The intoxication resulted in abnormal intracellular deposits of fat in most tissues and organs.

These deposits are revealed by general fat stains, such as Sudan Black and Scharlach R., on frozen sections of formaldehyde-fixed material. We have studied, by the available histochemical methods, the composition of these fats in liver, striated muscle, nervous cells, kidney, uterus epithelium and histiocytes. The fat droplets have no colour of their own, which shows that they do not belong to the carotinoid and chromo-lipoid groups. They are isotropic in polarized light. The Liebermann reaction gives a negative result, enabling us to exclude the presence of cholesterol and cholesterides, at least in notable quantities, except in some extracellular deposits in the lumen of terminal collecting tubules of the kidneys. The absence of free cholesterol is confirmed by a negative Windaus digitonine reaction. The Smith–Dietrich reaction for lipins (phospho- and galacto-lipins) is slightly positive. The Smith Nile Blue reaction for triolein is strongly positive; some fat droplets are red and appear to be constituted exclusively of triolein, but most droplets are violet, suggesting that the fat deposits are a mixture of saturated and unsaturated glycerids and of lipins. Since it has been denied that Fischler's method has any real value, there is no histochemical reaction for fatty acids.

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