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Uptake of campesterol in pigeon intestine

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SUMMARY

Campesterol was the major plant sterol found in the gastrointestinal tract, liver, and plasma of pigeons fed diets containing large amounts of β-sitosterol and small amounts of campesterol. On feeding plant sterol mixtures, it was found that campesterol was taken up preferentially by the pigeon intestine.

Plant sterols have been shown to be absorbed to a limited extent in most animal species[1-3]. In most of these studies, however, β-sitosterol was used extensively and little attention was paid to the accompanying sterols, campesterol and stigmasterol. The hypocholesteremic potency of plant oils in chicks has been shown to be related to their campesterol content[4]. Two isolated studies, by Kuksis and Huang[5] and by Boorman and Fisher[6], suggested that campesterol (then called γ-sitosterol) might be better absorbed than β-sitosterol. However, the importance of these studies was not fully realized, and plant sterol mixtures containing 10 to 30% campesterol have been used as 'unabsorbable' markers for sterol balance studies[7]. In humans, no systematic studies of absorption of different plant sterols have been reported to date. During our studies with the White Carneau pigeon, a breed in which atherosclerosis occurs spontaneously, we[8] noticed that the predominant plant sterol in the bile was campesterol, although the diet contained small amounts of campesterol and large amounts of β-sitosterol. This stimulated us to determine the ratios of these sterols in various tissues of this species.

The White Carneau pigeons, obtained from the Palmetto Pigeon Plant (Sumter, S.C.), were 4 to 6 years old and were fed a mixed grain diet (Purina Pigeon Chow, Ralston Purina Co., St. Louis, Mo.). The pigeons were killed after a blood sample had been obtained. The liver and gastrointestinal tract were then excised. Segments of the gastrointestinal tract were divided and washed free of the intestinal contents with saline and 1 mM sodium taurodeoxycholate. Bile was collected from some of the pigeons after construction of a bile fistula. The total lipids of the tissues were extracted by the procedure of Folch *et al.*[9]. Total lipid extracts and bile were saponified and the sterols were purified by thin-layer chromatography[8,10].

After elution the sterols were quantitated by gas–liquid chromatography as their silyl ethers, using cholestane as an internal standard[8]. Identification of the sterols was based on their behavior in gas–liquid chromatography as trimethyl silyl ethers on 3.8% W-98 on Diatoport S (100–120 mesh) and as trifluoroacetates[11] on 1% QF-1 on Gas Chrom Q (100–120 mesh). The retention times were compared to the retention times of authentic standards (Applied Science Laboratories, State College, Pa., and Mann Research Laboratories, New York, N.Y.). Pure samples of campesterol and β-sitosterol were kindly furnished by Dr. M.J. Thompson (Agricultural Research Station, Beltsville, Md.). During the procedure the recoveries were checked by using [4-^{14}C] cholesterol (specific activity, 60.9 mC/mole) from New England Nuclear Corporation (Boston, Mass.).

The pigeon diet contained mainly β-sitosterol (74.9%) and smaller amounts of campesterol (17%) and stigmasterol (8.1%) (Fig. 1A). On gas–liquid chromatography, the retention times (relative to cholestane) for the trimethyl silyl ethers of campesterol, stigmasterol, and β-sitosterol on 3.8% W-98 columns were 3.16, 3.47, and 4.05, respectively. The plant sterol composition of the intestinal contents was similar to that of the diet. The sterols of the proximal part of the intestine itself, however, contained predominantly cholesterol (98%) and plant sterols (2%) (Fig. 1B). The ratio of campesterol to β-sitosterol was increased 10-fold (to 2.34) in contrast to that in the diet, indicating a preferential uptake of campesterol. The possible contribution of 4-methyl sterols to the campesterol peak was ruled out on the basis that these sterols have a faster migration (R_F 1.06 relative to campesterol) on thin-layer chromatographic separation done prior to gas chromatography. Since there is no difference in the distribution of cholesterol and plant sterols between various intestinal phases[7], the increased absorbability noted previously[5,6] could be due to increased uptake by the mucosa. The plant sterol ratios in the duodenum and distal part of the intestine similarly showed an increased amount of campesterol. The average campesterol to β-sitosterol ratio in the distal part of the intestine (3.92) was higher than that of the proximal intestine (2.34), duodenum (3.16), and stomach (1.23).

The mean concentration of plant sterols in various parts of the intestinal tract (per g) were: proximal intestinal segment, 28.45 μg; distal intestinal segment, 19.35 μg; duodenum, 13.43 μg; and stomach, 13.83 μg. The presence of plant sterols in the distal part of the intestine indicates that some absorption of these sterols occurs here. Stigmasterol was present only in trace quantities in the various segments of the intestine and in plasma, although in the diet its concentration was half that of campesterol. This indicates that, of the three plant sterols, campesterol is preferentially taken up by pigeon intestinal mucosa. The preferential uptake of campesterol by intestinal mucosa was confirmed by feeding 1 g of Cytellin (a mixture of 65% β-sitosterol, 30% campesterol, and 5% stigmasterol) to fasted pigeons and analyzing the plant sterol ratios in the intestine (Fig. 1C) after 4 h. The mean campesterol to β-sitosterol ratio in the small intestine was 1.50, in contrast to 0.46 in the fed mixture.

The possibility that the decreased proportion of β-sitosterol in the intestinal segments might be due to an increase in its transfer to the blood was excluded by analyzing the plant sterol content of the plasma (Fig. 1D). The average ratio of campesterol to β-sitosterol in the plasma of the pigeons was 5.3. After the β-sitosterol-rich diet, no increase in β-sitosterol concentration in the plasma was noted at any time. This indicates that its uptake and successive transfer into the systemic circulation of the pigeon is less

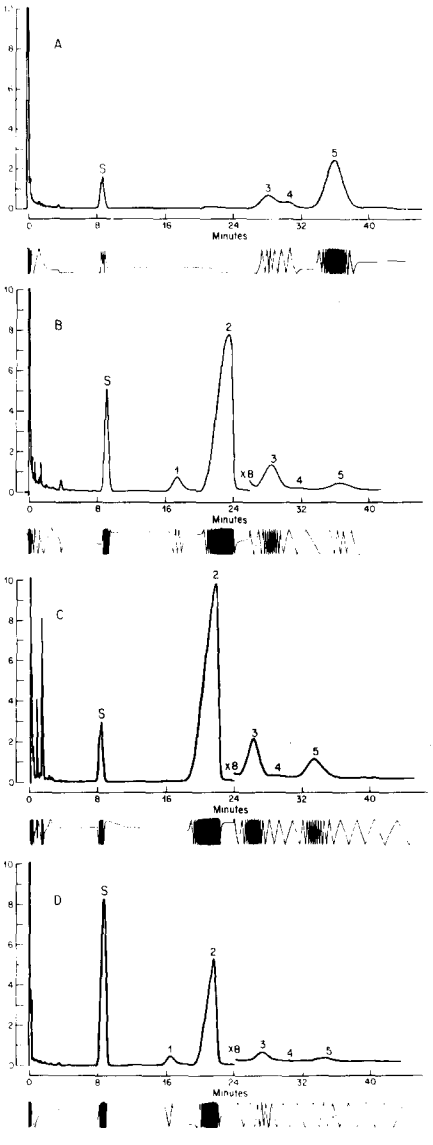

Fig. 1. Gas–liquid chromatographic analysis of sterols. A. Dietary sterols. B. Sterols of proximal intestinal segment of pigeons (fed diets containing sterol mixture shown in A). C. Sterols of proximal intestinal segment of pigeons (fed Cytellin containing 65% β-sitosterol, 30% campesterol, and 5% stigmasterol). D. Plasma sterols of pigeons (fed diet containing sterol mixture shown in A). Peak identification: 1, unknown; 2, cholesterol; 3, campesterol; 4, stigmasterol; 5, β-sitosterol. Peak S in all chromatograms represents internal standard, 5α-cholestane. Sterols were chromatographed as trimethyl silyl ethers. Column conditions are described in the text.

than that of campesterol. The possibility that β-sitosterol is metabolized to undetectable compounds in the intestinal mucosa is not ruled out in the present study. This, however, is less likely since such specific sterol alteration has not been hitherto reported. Liver and bile

of these pigeons showed similar campesterol to β-sitosterol ratios (4.1 to 4.9), confirming the preferential entrance of campesterol. The campesterol to β-sitosterol ratio in the plasma and liver of these animals was nearly twice as high as that in the intestine, indicating that another step of specificity of preferential campesterol entry into the system might lie in the transfer of this sterol from the intestinal cells into the circulation. Recently, in a human subject fed a 2:1 mixture of β-sitosterol and campesterol, the ratio of these sterols in the bile at 12 and 36 h was 1:1 (M.T.R. Subbiah, B.A. Kottke and M.A. Carey, unpublished observations, 1971).

These studies indicate that the intestinal mucosa in animals is capable of discriminating among various plant sterols and that great caution should be exercised in extrapolating absorption data from studies using plant sterol mixtures. This is of particular importance in view of the fact that plant sterols are degraded into a variety of products in animal tissues[12].

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