INHIBITION OF β-SITOSTEROL ON INTESTINAL
CHESTEROL ABSORPTION IN RAT USING
IN VIVO DUAL ISOTOPE RATIO METHOD

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Summary The inhibitory effects of β-sitosterol on intestinal chole-
sterol absorption were studied by means of a dual isotope plasma ratio
method (in vivo), which is a new technique for the measurement of
cholesterol absorption, as well as a ligated-loop method (in situ). The
results obtained were as follows:
1. The absorption of β-sitosterol itself was significantly less than
cholesterol. Cholesterol was selectively absorbed from rat intestine.
2. When 100 to 1,000 μg of β-sitosterol were added to the dose solution
containing 10 μg of cholesterol, cholesterol absorption by the in vivo
experiment decreased with the increase of additional β-sitosterol.
3. A similar inhibitory effect of β-sitosterol was observed by the in situ
ligated-loop method.

These results suggest that β-sitosterol actually inhibits cholesterol
absorption in the physiological state of an animal.

Keywords dual isotope, ligated-loop, selective absorption, β-sitosterol,
cholesterol, intestinal (absorption), inhibition

Some plant sterols, such as β-sitosterol and stigmasterol, are thought of as
agents preventing hypercholesterolemia. The lowering effect of dietary β-sitosterol
on serum cholesterol of rat (1), chick (2) and man (3, 4) suggest that β-sitosterol
may inhibit the intestinal absorption of cholesterol. Mattson et al. (5) originally
showed the effect of β-sitosterol in rats with lymph-duct fistula, which gave a direct
estimation of cholesterol absorption from the intestinal tract. However, Sylven and
Borgström demonstrated contradictory results on intestinal absorption of choles-
terol and β-sitosterol (6) using the same methods. It is not yet clear whether the

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inhibitory effect of β-sitosterol would actually occur on the intestinal absorbing process of cholesterol or not.

Recently, Zilversmit et al. have developed an in vivo dual isotope plasma ratio method as a new technique for the measurement of cholesterol absorption in rat (7, 8). The method has been evaluated as very reliable and useful in the physiological state of animals, and even on man (9). Therefore, it seemed interesting to investigate the effect of β-sitosterol on cholesterol absorption using the dual isotope ratio method.

The present paper describes the β-sitosterol effect on intestinal cholesterol absorption by not only the in vivo dual isotope ratio method but the in situ ligated-loop technique.

**METHODS**

1. **Chemicals and animals.** 7-[3H]-Cholesterol (s.a. 21.5 Ci/mmol) was obtained from New England Nuclear Co. (Boston, MA), and 4-[14C]-cholesterol (s.a. 0.057 Ci/mmol), 22,23-[3H]-β-sitosterol (s.a. 58 Ci/mmol) and 4-[14C]-β-sitosterol (0.053 Ci/mmol) were obtained from Amersham/Searle Co. (Arlington Height, IL). The purity of these radioactive sterols was tested by thin layer chromatography on silica gel G (Merck Co., Darmstadt, Germany) with the solvent system of benzene: ethylacetate = 2:1 (v/v). Sterols with a purity of more than 93% were used in all experiments. The micellar dose solutions containing radioactive cholesterol (1.0 μCi/0.5 ml) or β-sitosterol (1.0 μCi/0.5 ml) were prepared by dissolving various amounts of the corresponding non-radioactive sterol and 5 mM glycerol monooleate (Sigma Chem. Co., St. Louis, MO) in 50 mM sodium phosphate buffer (pH 6.3) containing 5 mM glycochenodeoxycholic acid (Tokyo Tanabe Co., Tokyo). These solutions were incubated at 37°C overnight to produce stable micellar particles.

Sprague-Dawley rats weighing 250–300 g were fed with a commercial chow (Oriental Yeast Co., Tokyo) ad libitum, and fasted for 24 hr before the experiments.

2. **Dual isotope plasma ratio method (in vivo).** The measurement of cholesterol and β-sitosterol absorption from the intestine were carried out by the dual isotope ratio method (8, 9) with slight modifications as follows; under light anesthesia with ether, the abdominal cavity was opened by incision, and 1.0 μCi of [14C]-cholesterol or [14C]-β-sitosterol in 0.5 ml of micellar dose solution was administered into the duodenum. Immediately after the dosage, 1.0 μCi of [3H]-cholesterol or [3H]-β-sitosterol dispersed in 0.5 ml of serum obtained previously from the same animal was also injected into the left external jugular vein (I.V. dose). Thereafter, the rat was fed with the same diet and water ad libitum in a wire cage, and 2 ml of blood were collected from the right external juglar vein of the rat with heparinized syringes at 48, 72 and 96 hr. The radioactive sterols were extracted from the plasma samples (1 ml each) with 3 ml of ethanol: hexane = 2:1 (v/v) after saponification with 50% KOH at 50°C for 30 min. Both radioactivities of [3H]- and
[14C]-sterol were then determined simultaneously in a toluene-based scintillation
cocktail containing 0.4% Omnifluor (New England Nuclear Co., Boston, MA) by a
Packard Tri-Carb liquid scintillation spectrometer, Model 3380 (Packard Co.,
Downers Grove, IL). Percent absorption of cholesterol or β-sitosterol was
calculated by the formula as follows:

\[
\% \text{ absorption} = \frac{[\text{plasma } [14C] \text{ (DPM)}]/[\text{dosed } [14C] \text{ (DPM)}]}{[\text{plasma } [3H] \text{ (DPM)}]/[\text{dosed } [3H] \text{ (DPM)}]} \times 100
\]

3. Ligated-loop method (in situ). The measurements for intestinal mucosal
uptake of sterols were carried out by the (in situ) ligated-loop method of
Sylven (10). After an animal was anesthetized with nembutal (30 mg/kg), 4 to 6
segments of 4-cm length were made in the jejunum of an animal with an intact
current of blood and lymph. One-hundredth μCi of [14C]-cholesterol or [3H]-β-
sitosterol in 0.5 ml of dose solution was injected into each segment, and then
incubated in the body for 30 min after the abdominal wall had been closed. At the
end of the incubation, each segment of the loop was washed with 2 ml of ice-chilled
physiological saline. Then, the tissue was further washed 5 times with 2 ml of ice-
chilled 5 mM sodium deoxycholate in saline to remove the sterols that remained on
the surface of the mucosa. Finally, each washed tissue was weighed (200-400 mg),
then the radioactive sterols were extracted with 9 ml of chloroform:methanol =
2:1 (v/v), as described by Folch et al. (11). After the solvent had dried, the
radioactivities were determined, and the amount of sterol incorporated into the
tissue was calculated and expressed as μg/100 mg tissue/30 min.

RESULTS

1. Selective absorption of cholesterol and β-sitosterol
β-Sitosterol absorption was measured by the dual isotope ratio method, since
different results on intestinal absorption of β-sitosterol have been reported by some
laboratories using other methods such as a balance study (12, 13) and lymph
collection (6). The absorption ratio of β-sitosterol was 5.4 ± 1.6% (in 500 μg dose)
and that of cholesterol was 44.2 ± 3.8% (in 500 μg dose) (Table 1). These results
show that β-sitosterol is resistant to intestinal absorption, and cholesterol is
selectively absorbed from rat intestine.

2. Effect of β-sitosterol on the absorption and mucosal uptake of cholesterol
In order to observe the β-sitosterol effect on cholesterol absorption, 100 to
1,000 μg of β-sitosterol (Gaskuro Co., Tokyo, purity 98%) were added to 10 μg of
[14C]-cholesterol (1.0 μCi) in a 0.5 ml dose solution, and cholesterol absorption was
measured by the dual isotope ratio method. As shown at left in Fig. 1, cholesterol
absorption clearly decreased with an increase of additional β-sitosterol. The
absorption ratio of cholesterol was about 48.3% of control in the presence of
Table 1. The selective absorption of cholesterol and \( \beta \)-sitosterol.

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<th>Cholesterol</th>
<th>( \beta )-Sitosterol</th>
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<tr>
<td>DIRM (in vivo)</td>
<td>44.2 ± 3.8(^a)</td>
<td>5.4 ± 1.6(^a)</td>
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<tr>
<td>Ligated-loop (in situ)</td>
<td>10.3 ± 2.7(^b)</td>
<td>3.5 ± 0.7(^b)</td>
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Five hundred \( \mu g \) of [\( ^{14}C \)]-cholesterol or [\( ^{14}C \)]-\( \beta \)-sitosterol in a 0.5 ml dose solution were given into the duodenum and \( \% \) absorption of sterol was measured by DIRM as described elsewhere. In the *in situ* ligated-loop method, 100 \( \mu g \) of [\( ^{14}C \)]-cholesterol or [\( ^{3}H \)]-\( \beta \)-sitosterol in a 0.5 ml of dose solution were injected into the jejunal segment and the amount of sterol incorporated in mucosa was measured. The values were expressed as \( \mu g \) of sterol per 100 mg tissue per 30 min incubation.

\(^a\) \% absorption per rat. \(^b\) \( \mu g \) of sterol incorporated per 100 mg tissue per 30 min.

**Fig. 1.** Inhibitory effect of \( \beta \)-sitosterol on cholesterol absorption and mucosal uptake.

The cholesterol absorption ratio in the administration of 10 \( \mu g \) of cholesterol in a 0.5 ml dose solution was measured by DIRM in the presence of 0 (control), 100, 500 and 1,000 \( \mu g \) of \( \beta \)-sitosterol (left in Fig. 1). The values are expressed as \( \% \) of control. Cholesterol uptake into the jejunal mucosa of a segment was measured by the *in situ* ligated-loop method in the presence of 0 (control), 10, 100, 200 and 500 \( \mu g \) of \( \beta \)-sitosterol (right in Fig. 1). Cholesterol given into a segment was 10 \( \mu g \) in a 0.5 ml dose solution. The values (\( \mu g \) of incorporated cholesterol/100 mg tissue/30 min) were also expressed as \( \% \) of control.

1,000 \( \mu g \) of \( \beta \)-sitosterol (100 times the amount of cholesterol). The \( \beta \)-sitosterol effect on cholesterol absorption was also studied by the ligated-loop technique.

When 10 to 500 µg of β-sitosterol were added to the dose solution containing 10 µg of cholesterol, the mucosal uptake of cholesterol decreased with an increase of additional β-sitosterol (right in Fig. 1). The cholesterol uptake was about 36% of the control in the presence of 500 µg of β-sitosterol (50 times the amount of cholesterol).

These results strongly indicate that β-sitosterol has an inhibitory effect on intestinal cholesterol absorption. Furthermore, the results support that both the methods, in vivo dual isotope ratio method and in situ ligated-loop method, bring similar results of intestinal absorption and mucosal uptake of cholesterol.

**DISCUSSION**

Sylven and Borgström (6) have demonstrated that β-sitosterol showed no inhibitory effect on cholesterol absorption in rat with lymph duct fistulae. However, the result seems to occur in animals in which cholesterol metabolism might lead to an unsteady state during the long-term collection of lymph. Actually, the absolute value of absorbed cholesterol in their experiment was much lower than that of other groups (5, 14). On the other hand, Mattson et al. (5) showed the inhibitory effect of β-sitosterol in rats with lymph duct fistulae when an excess amount of β-sitosterol (more than 30 mg per rat) was administered. The inhibitory effect of β-sitosterol observed in their experiment may be related to the reduction in cholesterol solubility to disturb the micell formation in a stage before absorption. However, our results also indicated that cholesterol absorption and mucosal uptake were decreased in the presence of β-sitosterol, in spite of the total sterol concentration being much lower (3 mM or less in 5 mM bile acid) as Borgström has reported (15). In our results, β-sitosterol seems to have other inhibitory mechanisms than the reduction in cholesterol solubility on intestinal cholesterol absorption. Furthermore, similar inhibitory effects of β-sitosterol observed by both the in vivo dual isotope ratio method and in situ ligated-loop technique appears to suggest that the inhibition takes place at the stage of mucosal uptake in the absorption process of cholesterol. As β-sitosterol actually led to a decrease in cholesterol uptake in the in situ experiment, while the cholesterol incorporated into mucosa was not detected in the lymph or blood during the 30-min incubation. The selective absorption of sterols would also be related to the stage of mucosal uptake as suggested elsewhere (16).

From the present study, it was demonstrated that β-sitosterol inhibited the absorption of cholesterol in the physiological state. However, further observations are necessary to clarify the mechanism of the inhibitory effect of β-sitosterol on cholesterol absorption.

**REFERENCES**


