nylosuccinate production are increased, the operation of the PNC must be favored. This would require an activation of AMP deaminase sufficient to produce significant amounts of IMP, without establishing cellular conditions which would inhibit adenylosuccinate synthetase. These circumstances may arise when the rate of ATP hydrolysis is increasing. Hence, the purine nucleotide cycle may be important as anaplerotic process mainly in the working muscle when the ATP level drops. This conclusion is in accordance with the experiments published recently by Aragon and Lowenstein[6].

It should be emphasized that the pyruvate carboxylase activity in rat skeletal muscle is controlled by the ATP/ADP ratio[4]. With a decreasing ATP/ADP ratio, a decrease of the enzyme activity was observed. This suggests that, when ATP level decreases the pyruvate carboxylase activity might be suppressed.

Considering the kinetic properties of malic enzyme from liver[6] and heart mitochondria[4], some authors deny the possibility that the pyruvate carboxylation catalyzed by the malic enzyme could be an important process responsible for the supply of the Krebs cycle intermediates in skeletal muscle. Our results reported recently suggest that pyruvate carboxylation catalyzed by extramitochondrial malic enzyme can increase the level of the citric acid cycle intermediates if the concentration of pyruvate is increased in the tissue. It seems likely, therefore, that when the ATP and pyruvate levels are low in skeletal muscle the PNC is responsible for a substantial part if not for the whole of the increase of the citric acid cycle intermediates.

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Effects of plant sterols on cholesterol concentration in the rat small intestine[1]

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Summary. The effects of feeding single doses of β-sitosterol, campesterol and stigmasterol on cholesterol concentration in the rat small intestine was studied to clarify their roles in cholesterol absorption. The different plant sterols affected free and ester cholesterol concentrations differently in the different intestinal segments suggesting that they have different effects on such intestinal processes as uptake, esterification and possibly synthesis of cholesterol and transport of cholesterol esters out of the mucosal cells into the lymphatics.

β-Sitosterol, campesterol and stigmasterol, the commonly found plant sterols are usually considered together as if they are one single entity. However, in animals and humans the absorption of these sterols are different[2-7] and also both in vivo and in vitro studies have shown that the uptake of these sterols by the rat small intestine is different[8]. It was of interest, therefore, to study the effects of plant sterols individually on cholesterol concentration in the small intestine to clarify their roles on the intestinal processes involved in cholesterol absorption such as uptake and esterification because the well-known hypocholesterolemic effect of 'plant sterols'[5,9,10] may be mediated by their actions on such processes.

Materials and methods. Sitosterol, campesterol and stigmasterol (> 99.5% pure, Applied Science Labs, State College, PA) were used without further purification. Adult male and female Sprague-Dawley rats, each weighing 250-300 g, were fed the commercial pellet diet (Ralston Purina Co., St. Louis, MO) until used. They were fasted for 24 h and under light ether anesthesia, were fed by stomach tube about 2 g olive oil in which the individual plant sterols were dissolved. The composition of the test meals is given in table 1. The control animals were fed the oil only. All animals were fed at about the same time, 9.30 h on the day of the experiment. 4 h later, animals were killed and the small intestine was dissected and collected in ice-cold saline. The intestine was flushed thoroughly with saline, opened longitudinally, again washed thoroughly with saline, and divided into 6 serial segments of about equal length and dried to a constant weight in a heated vacuum

Table 1. Plant sterol concentrations in the test meals

Test meal	Plant sterols, mg/g oil			
	β-Sitosterol	Campesterol	Stigmasterol	Total
Oil	0.27 (65.9)*	0.14 (34.1)	trace**	0.41
Oil + β-sitosterol	10.16 (98.8)	0.12 (1.2)	trace	10.28
Oil + campesterol	0.23 (2.8)	7.98 (97.2)	trace	8.21
Oil + stigmasterol	0.26 (3.5)	0.15 (2.0)	7.08 (94.5)	7.49

* Figures in parentheses are percent of total; ** trace indicates < 0.01 mg/g.

dessicator at 80 °C. The lipids were extracted by the method of Folch et al.[11] and the extract was made up to 10 ml. Aliquots were taken for total, free, and esterified cholesterol determinations by TLC and GLC[8]. The GLC method used clearly separated cholesterol from plant sterols and other possible sterol contaminants.

Total cholesterol in the lipid extracts were extracted with hexane after saponification with alcoholic KOH by the method of Abell et al.[12]. The hexane extract was evaporated to dryness under N_2 and subjected to GLC as trimethyl silyl ether derivative with 5 α-cholestane used as the internal standard. Free and esterified cholesterol were separated by TLC and the free and ester bands were scraped and eluted with diethyl ether. The free cholesterol was subjected to GLC as above and cholesterol esters were saponified, extracted, and processed as the free sterol.

Data were analyzed for statistical significance by the t-test of difference between means between experimentals and controls.

Results. Cholesterol concentration in the proximal intestine was significantly higher by 20% in the stigmasterol-fed group compared to controls, but in the other 2 groups no differences were found (table 2). Similar results were obtained in the middle segment, but in the distal segment, cholesterol concentration was higher by 42, 38, and 55% in all 3 groups compared to controls.

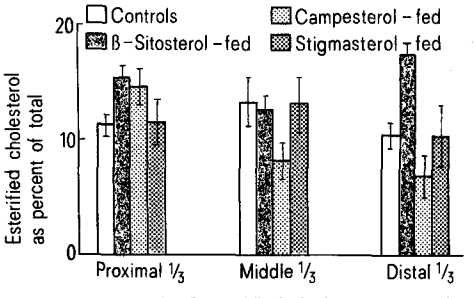
The percent esterified cholesterol of the proximal segment was higher by 34 and 30% in the sitosterol- and campesterol-fed groups compared to controls, but in the stigmasterol-fed group the values were similar to controls (fig.). In the middle segment, only in the campesterol-fed group, the percent ester cholesterol was lower by 44% than the controls. In the distal segment, compared to controls, the percent ester cholesterol was higher by 68% in the sitosterol-fed but was lower by 34% in the campesterol-fed group.

Cholesterol concentration was significantly higher in all 3 experimental groups by 30%, 20% and 27% in the distal than in the middle segment (table 2). The percent ester cholesterol was lower by 17 and 44% in the middle than in the proximal segment in the sitosterol- and campesterol-fed groups respectively. In the distal segment percent ester cholesterol was higher by 40% than in the middle segment only in the sitosterol-fed group. Also, only in the campesterol-fed group, the percent esterified cholesterol was lower by 53% in the distal than in the proximal segment.

Discussion. Feeding single doses of β-sitosterol, campesterol and stigmasterol, the 3 commonly found plant sterols, affected total, free and esterified cholesterol concentrations differently in the different segments of the rat small intestine. Since stigmasterol is not taken up by the mucosal cells as we have shown previously both in vivo and in vitro[8] and hence can have no effect on the intracellular processes such as esterification, synthesis, incorporation into chylomicrons and transport, the increase in cholesterol concentration in the different segments of the intestine (table 2) must have occurred because of increased uptake of cholesterol by the mucosal cells. The mechanism of this increased uptake of cholesterol by the mucosal cells is not clearly understood. Before the uptake of the cholesterol by the mucosal cells can take place by passive diffusion from mixed micelles formed within the intestinal lumen in the presence of bile acids[13], cholesterol must partition out of mixed micelles into monomeric form. Possibly stigmasterol enhances the partitioning of cholesterol, thereby increasing the availability of cholesterol in the form suitable for uptake by the mucosal cells.

β-Sitosterol and campesterol apparently have no effect on cholesterol uptake by the mucosal cells in the proximal and middle segments because no change in total cholesterol concentration occurred in these segments (table 2). In the distal segment, however, the increase may have occurrred as a result of increased uptake of cholesterol by the mucosal cells, or increased synthesis of cholesterol, or because these 2 sterols may have interferred with the transport of cholesterol out of the mucosal cells into the lymphatics.

The increase in esterified cholesterol in the proximal and distal segments of sitosterol-fed rats compared to controls



Mean percentages (± SE) of esterified cholesterol (EC) in different segments of small intestine of rats fed test meals containing plant sterols. In the proximal 1/3, percent EC was higher ($p < 0.025$) in β-sitosterol- and campesterol-fed groups than the respective controls. In the middle 1/3, percent EC was lower ($p < 0.01$) in campesterol-fed group than the respective controls. In the distal 1/3, percent EC was higher ($p < 0.001$) in β-sitosterol-fed group and lower ($p < 0.025$) in campesterol-fed group than the respective controls.

Table 2. Cholesterol concentration in small intestine of rats after feeding test meals

Intestinal segment	Controls Only oil fed	Group 1 β-Sitosterol fed	Group 2 Campesterol fed	Group 3 Stigmasterol fed
Proximal 1/3				
1	6.378 ± 0.444	7.225 ± 0.579	7.350 ± 0.666	8.175 ± 0.485
2	6.172 ± 0.651	6.200 ± 0.576	6.525 ± 1.161	6.950 ± 0.556
Mean ± SE	6.275 ± 0.373	6.712 ± 0.425	6.937 ± 0.639	7.562 ± 0.413[a]
Middle 1/3				
3	5.625 ± 0.448	5.700 ± 0.568	6.250 ± 0.659	6.350 ± 0.218
4	5.662 ± 0.375	6.075 ± 0.510	6.225 ± 0.936	6.875 ± 0.137
Mean ± SE	5.643 ± 0.276	5.887 ± 0.361	6.237 ± 0.530	6.612 ± 0.155[b]
Distal 1/3				
5	5.668 ± 0.711	7.350 ± 0.347	7.600 ± 0.547	8.050 ± 0.155
6	5.099 ± 0.221	7.975 ± 0.111	7.350 ± 0.284	8.725 ± 0.340
Mean ± SE	5.394 ± 0.364	7.662 ± 0.206[c]	7.475 ± 0.290[c]	8.387 ± 0.215[c]

Values are mean ± SE and expressed as mg/g dry wt tissue. There were 4 animals in each group. [a] Significantly different from the respective control, $p < 0.05$; [b] significantly different from the respective control, $p < 0.025$; [c] significantly different from the respective control, $p < 0.001$.

(fig.) suggested a increase in the activities of the enzymes, cholesterol esterase and acylcoenzyme A cholesterol acyltransferase (ACAT), within the intestinal mucosa. The increase may also have occurred because of an inhibitory effect of sitosterol on the transport of ester cholesterol out of the cells. In the proximal segment the increase in esterified cholesterol occurred without concomitant increase in the total cholesterol suggesting that sitosterol has an inhibitory effect on the incorporation of cholesterol esters into chylomicrons. If both the processes, namely incorporation of cholesterol esters into chylomicrons and the transport of chylomicrons out of the mucosal cells, have not been interferred with, the increased amount of cholesterol ester formed should have been incorporated into chylomicrons and transported out of the cells, which would have caused not only a decrease in ester cholesterol but also a decrease in total cholesterol in the segment. In contrast, in the middle segment, sitosterol appears to have no effect on either uptake or the intracellular events, because neither total cholesterol nor cholesterol ester content in this segment differed from that in controls (table 2).

The effect of campesterol was similar to that observed for β-sitosterol in the proximal segment, but in the middle segment the decrease in ester cholesterol with no increase in total cholesterol (table and the fig.) suggested that campesterol inhibited the activities of cholesterol esterase and ACAT in the mucosal cells. In the distal segment, the higher total cholesterol and lower ester cholesterol content compared to controls suggested that the uptake of cholesterol was increased and the activity of the esterifying enzymes was inhibited at the same time, producing an accumulation of free cholesterol within the mucosal cells. Less esterification of cholesterol within the mucosal cells would also suggest a decrease in cholesterol absorption in this segment.

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Metal accumulation in *Agaricus bisporus*: Influence of Cd and Cu on growth and tyrosinase activity[1]

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Summary. To investigate heavy metal uptake in the common mushroom *Agaricus bisporus*, commercial cultures of the fungus were exposed to different amounts of copper ad cadmium. In contrast to copper, cadmium already exerted pronounced toxic effects at low concentrations (> 10 µM) in the compost and concomitantly enhanced the uptake of copper. Tyrosinase activity measured in the fruit bodies increased sharply, when small amounts of Cd (10 µM) were added to copper-rich compost. Gel filtration experiments with crude extracts from fruit bodies demonstrated the absence of a low molecular weight metal binding protein.

The accumulation of heavy metals in the common mushroom *Agaricus bisporus* has been the subject of numerous studies[3]. It was shown that addition of cadmium to the casing soil of commercial cultures increased the uptake of this metal[4]. Furthermore, cadmium was reported to enhance the growth of the cadmium-accumulating mushroom *Agaricus abruptibulbus* in liquid culture[5]. The effects of copper additions were not investigated although this metal is also taken up and is moreover known to play a vital role as a cofactor for various enzymes[6]. Furthermore it is still unclear in which form these metals occur intracellulary in higher mushrooms. In the ascomycete *Neurospora crassa* copper was demonstrated to be bound to a low molecular weight protein. From amino acid sequence data[7] it was established that this protein belongs to the metallothioneins, an ubiquitous class of cysteine- and metal-rich proteins[8].

Commercial cultures of *Agaricus bisporus* were grown according to standard cultivating methods[9]. Shortly after inoculation various amounts of cadmium (0-300 µm) and copper (0-1 mM) were added to the compost. The fruit bodies were harvested at constant size of the cups (⌀3 cm), lyophilized immediatly and weighed. The results are presented in figure 1. In agreement with the known toxicity of cadmium[10] the number of fruit bodies formed is strongly reduced and the growth rate declines at concentrations above 10 µM. On the other hand the growth is unaffected on addition of copper over a rather broad concentration range. Lyophilized cups, which are known to contain the major amount of heavy metals[11] were wet ashed[12] and the cadmium, copper and zinc content was measured by atomic absorption spectroscopy (Instrumentation Laboratory Inc. Il 157, air/acetylene flame). Both cadmium and copper are accumulated to about the same extent (figs 2 and 3). Cadmium administration brings about a striking stimulation of copper uptake (fig. 2) whereas the presence of copper has no influence on cadmium accumulation (fig. 3). Neither copper nor cadmium showed any effect on the uptake of zinc (data not shown). Thus we conclude that these metals could be transported via separate pathways