(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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2 K.N. Boorman and N. Fisher, Br. J. Nutr. *20*, 689 (1966).
3 A. Kuksis and T.C. Huang, Can. J. Biochem. Physiol. *40*, 1493 (1962).
4 R.G. Gould, R.J. Jones, G.V. LeRoy, R.W. Wissler and C.B. Taylor, Metabolism *18*, 652 (1969).
5 M.T.R. Subbiah, Am. J. clin. Nutr. *26*, 219 (1973).
6 A.K. Bhattacharyya and L.A. Lopez, Biochim. biophys. Acta *574*, 146 (1979).
7 A.K. Bhattacharyya and D.A. Eggen, Ann. Nutr. Metab. *25*, 85 (1981).
8 A.K. Bhattacharyya, Am. J. Physiol. *240*, G50 (1981).
9 D.W. Peterson, Proc. Soc. exp. Biol. Med. *78*, 143 (1951).
10 A.M. Lees, H.Y.I. Mok, R.S. Lees, M.A. McCluskey and S.M Grundy, Atherosclerosis *28*, 325 (1977).
11 J. Folch, M. Lees and G.H. Sloane-Stanley, J. biol. Chem. *226*, 497 (1957).
12 L.L. Abell, B.B. Levy, B.B. Brodie and F.E. Kendall, J. biol. Chem. *195*, 357 (1952).
13 A.B.R. Thompson, in: Disturbances in lipid and lipoprotein metabolism, p.29. Eds J. Dietschy, A.M. Gotto, Jr, and J.A. Ontko. Am. Physiol. Soc., Bethesda, MD 1977.

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

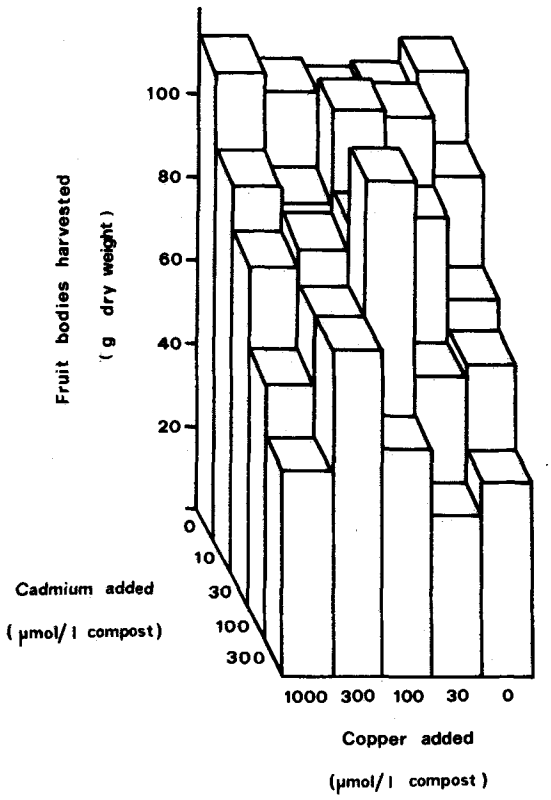

Figure 1. Fruit bodies harvested as a function of different amounts of copper and cadmium added to the compost*.

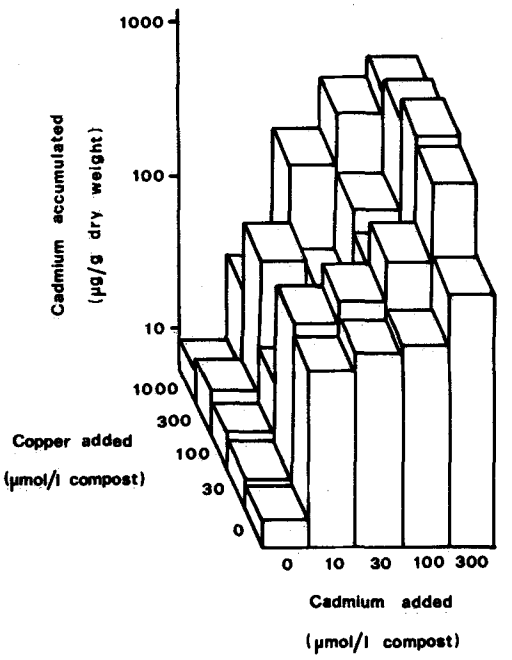

Figure 3. Cadmium accumulation in cups of *Agaricus bisporus* as a function of different amounts of cadmium and copper added to the compost*.

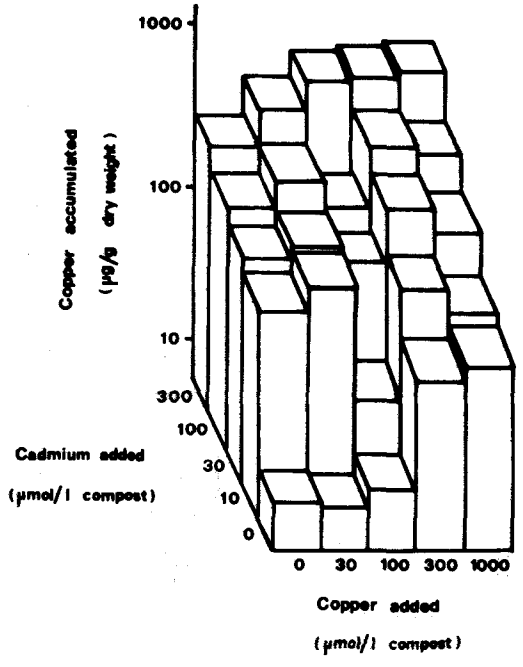

Figure 2. Copper accumulation in cups of *Agaricus bisporus* as a function of different amounts of copper and cadmium added to the compost*.

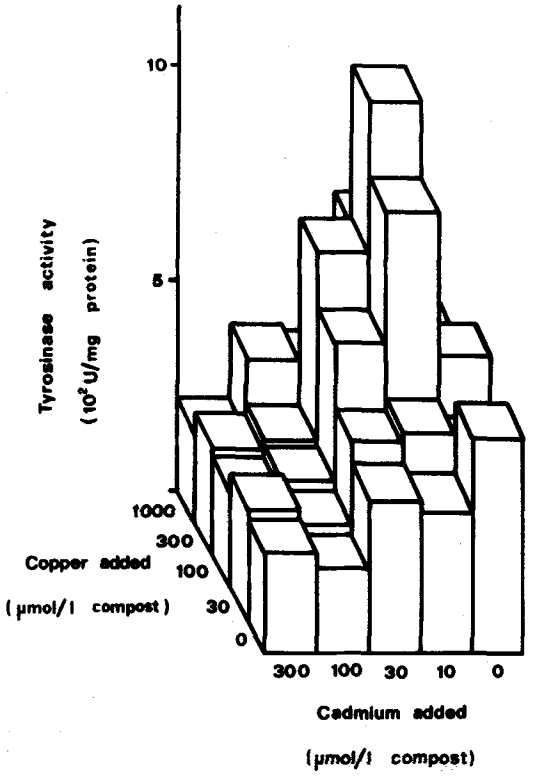

Figure 4. Tyrosinase activity in cups of *Agaricus bisporus* as a function of different amounts of copper and cadmium added to the compost*.

* Untreated compost contained the following metal concentrations of Zn (294 µM), Cu (144 µM) and Cd (< 2.5 µM) as determined by wet ashing and atomic absorption spectroscopy (see text).

but with a strong synergistic effect of cadmium uptake on that of copper.

Crude extracts of cup material were prepared by extraction with 0.1 M sodium phosphate pH 7.2 and centrifugation at 48,000 × g at 4 °C. Tyrosinase activity of the supernatants was determined according to Fox et al.[13]. The enzyme activity remains fairly constant upon addition of copper only to the compost. However, when small amounts of cadmium are added in addition, tyrosinase activity of samples grown on copper rich compost increases sharply (fig. 4). Toxic features of cadmium predominate however when higher amounts are offered. Concomitantly tyrosinase activity decreases and can no longer be restored by higher doses of copper.

In order to gain some insight into the possible storage form of the heavy metals, crude extracts (prepared as described above but with the buffer containing 10 mM thiourea to inhibit tyrosinase) were passed over Sephadex G-75 and G-50 columns. The elution profiles for cadmium, copper and zinc showed a minor peak eluting at V_0 and a major one at V_i of the columns. This result strongly suggests that neither cadmium, copper nor zinc are bound to a low molecular weight protein in *Agaricus bisporus* fruit bodies.

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3 T. Stijve and R. Besson, Chemosphere *2*, 151 (1976).
4 E. Laub, F. Waligorski and R. Woller, Z. Lebensmittelnunters. u. -Forsch. *164*, 269 (1977).
5 H.-U. Meisch, J.A. Schmitt and A.-R. Scholl, Naturwissenschaften *66*, 209 (1979).
6 E.I. Ochiai, in: Bioinorganic chemistry: An introduction, p.218. Allyn and Bacon, Boston 1977.
7 K. Lerch, Nature *284*, 368 (1980).
8 Y. Kojima and J.H.R. Kägi, Trends biochem. Sci. *3*, 90 (1978).
9 J. Lelley and F. Schmaus, Pilzanbau. Ulmer, Stuttgart 1976.
10 E.J. Underwood, in: Trace metals in human and animal nutrition, p.267. Academic Press, New York 1971.
11 R. Seeger, Z. Lebensmittelunters. u. -Forsch. *161*, 151 (1976).
12 R. Seeger, At. Absorption Newslett. *15*, 45 (1976).
13 A.S. Fox and J.B. Burnett, Proc. Soc. exp. Biol. *98*, 110 (1958).

Effects of 4-methyl-2-methylenevalerate, a non-metabolizable analogue of 2-ketoisocaproate, on insulin secretion and metabolism in *ob/ob* mouse pancreatic islets

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Summary. To determine the importance of 2-ketoisocaproate metabolism in its insulin secretory action, 4-methyl-2-methylenevalerate, a non-metabolizable analogue, was tested for its ability to promote insulin secretion, and to interfere with the metabolism and insulin secretory action of 2-ketoisocaproate. 4-Methyl-2-methylenevalerate did not induce insulin release by isolated *ob/ob* mouse pancreatic islets, but it inhibited insulin release in response to 2-ketoisocaproate and reduced the rate of decarboxylation and oxidation of labeled 2-ketoisocaproate. The results suggest that 4-methyl-2-methylenevalerate interferes with the insulin secretory action of 2-ketoisocaproate, owing to their common brached-chain chemical structure.

2-Ketoisocaproate is a potent stimulator of insulin release and is vigorously metabolized by isolated pancreatic islets[3,4]. 2-Ketoisocaproate has also been shown to increase insulin levels in the circulation in man[5], and this can explain the occurrence of hypoglycemia in patients with maple sirup urine disease, who have increased serum levels of 2-ketoisocaproate in the millimolar concentration range[6]. In order to determine the importance of metabolism of 2-ketoisocaproate for its insulin secretory action, 4-methyl-2-methylenevalerate was synthesized with the intention of preparing a non-metabolizable analogue of 2-ketoisocaproate with close structural similarity, by replacement of the double-bonded oxygen in the 2-keto group by a double-bonded methylene group. 4-Methyl-2-methylenevalerate was tested for its ability to induce insulin secretion and to interfere with metabolism and insulin secretory potency of 2-ketoisocaproate.

Materials and methods. 24-h starved adult non-inbred *ob/ob* mice (6-10 months of age) of either sex taken from a colony originating from Jackson Memorial Laboratories, Bar Harbor, MA, USA, and now bred in our department, were used in the experiments. Pancreatic islets were microdissected freehand from the *ob/ob* mouse pancreas according to Hellerström[7]. All islet incubation studies were performed at 37 °C in Hepes-buffered Krebs-Ringer bicarbonate media equilibrated with ambient air. Islets were freeze-dried and weighed on a quartz fiber balance. Insulin was determined radioimmunologically[8]. For determination of insulin release 2 microdissected islets were incubated for 60 min in 300 µl incubation medium supplemented with the test agents as described[4]. For determination of decarboxylation and oxidation rates of radioactively labeled 2-ketoisocaproate 10 microdissected islets were incubated for 60 min in 40 µl incubation medium with 2-keto[1-^{14}C] isocaproate (0.9 or 0.2 Ci/mole) or [U-^{14}C]2-ketoisocaproate (1.2 or 0.4 Ci/mole) as previously described[4]. 2-keto[1-^{14}C]isocaproate and [U-^{14}C]2-ketoisocaproate were prepared by oxidative deamination from L-[1-^{14}C]-leucine and L-[U-^{14}C]leucine respectively, as already described[4]. Byk-Gulden-Lomberg Chemische Fabrik, Konstanz, FRG, kindly provided 4-methyl-2-methylenevalerate. Other chemicals were obtained from the same sources as in earlier experiments[4].

Results. 4-Methyl-2-methylenevalerate did not induce significant insulin release by isolated incubated *ob/ob* mouse pancreatic islets at 5 mM and 20 mM concentrations (table). However, 4-methyl-2-methylenevalerate (20 mM) significantly inhibited 2-ketoisocaproate-induced insulin