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.

1 Acknowledgments. This work was supported by the Schweizerischer Nationalfonds grant 3.420-0.78 (to K.L.) and by Hauser Champignonkulturen AG. We are indepted to Dr D. Rast for helpful discussions.
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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

Effects of 4-methyl-2-methylenevalerate on insulin release in the absence and presence of 2-ketoisocaproate and on decarboxylation of 2-keto[1-^{14}C]isocaproate and oxidation of [U-^{14}C]2-ketoisocaproate by isolated incubated ob/ob mouse pancreatic islets

2-Ketoisocaproate	4-Methyl-2-methylenevalerate	Insulin release rate	Decarboxylation rate of 2-keto[1-^{14}C]isocaproate	Oxidation rate of [U-^{14}C]2-ketoisocaproate
mM	mM	ng/μg dry weight/h	mmoles/kg dry weight/h	mmoles/kg dry weight/h
None	None	1.81 ± 0.50 (15)		
None	5	2.50 ± 0.45 (15)		
None	20	2.54 ± 0.38 (15)		
5	None	6.30 ± 1.48 (15)	11.70 ± 0.83 (5)	8.71 ± 2.23 (6)
5	5	5.40 ± 0.89 (15)	11.35 ± 1.77 (5)	6.74 ± 1.16 (6)*
5	20	3.75 ± 0.55 (15)***	10.07 ± 0.96 (5)**	4.05 ± 0.48 (6)
20	None	7.06 ± 1.17 (15)	16.03 ± 1.80 (5)	7.11 ± 1.23 (6)
20	5	6.74 ± 1.01 (15)	10.73 ± 0.96 (5)	7.14 ± 1.36 (6)*
20	20	3.30 ± 0.84 (15)***	11.25 ± 1.19 (5)**	4.30 ± 0.56 (6)

Results are presented as the means ± SEM for the numbers of experiments given in parenthesis. The influence of 4-methyl-2-methylenevalerate (5 and 20 mM) on the effects of 2-ketoisocaproate on pancreatic islet function was tested for statistical significance with the analysis of variance. *$p < 0.10$; **$p < 0.05$; ***$p < 0.025$.

release by isolated incubated *ob/ob* mouse pancreatic islets (table). This inhibitory effect of 4-methyl-2-methylenevalerate on insulin release was paralleled by a significant inhibition of decarboxylation of 2-keto[1-^{14}C]isocaproate and of oxidation of [U-^{14}C]2-ketoisocaproate (table).

Discussion. A non-metabolizable analogue of 2-ketoisocaproate like 4-methyl-2-methylenevalerate, even if structurally closely similar, apparently loses its insulin secretory potency along with the replacement of the 2-keto group. Modifications of the chemical structure under maintenance of insulin secretory action are apparently not possible when these modifications go along with a loss of the substrate character. However, the non-metabolizable analogue 4-methyl-2-methylenevalerate may retain its ability to interact with the recognition mechanism of the keto acid 2-ketoisocaproate, as evidenced by its ability to inhibit 2-ketoisocaproate-induced insulin release. A feasible explanation for the inhibitory action of 4-methyl-2-methylenevalerate on 2-ketoisocaproate-induced insulin release is their common branched-chain chemical structure. Such an interpretation would also be in accordance with the finding by Hutton, Sener and Malaisse[9] that the branched-chain amino acids L-valine and L-isoleucine, but not the straight-chain amino acids L-norvaline and L-norleucine, significantly inhibited 2-ketoisocaproate-induced insulin release. These authors attributed the inhibitory action of the branched-chain amino acids on 2-ketoisocaproate-induced insulin release to the decreased availability of 2-ketoisocaproate due to transamination, though there was no comparable difference in the transamination rates of branched-chain and straight-chain amino acids by pancreatic islets[9].

1 The skilful technical assistance of Miss S. Detels is gratefully acknowledged.
2 This work was supported by grants from the Deutsche Forschungsgemeinschaft (Le 348/3 and Pa 229/2).
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Changes in the G6PDH/6PGDH ratio in the chick brain during development[1]

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Summary. The profile of the G6PDH/6PGDH ratio at various stages of development was drawn on the basis of the specific activities of glucose-6-phosphate dehydrogenase (G6PDH) and 6-phosphogluconic dehydrogenase (6PGDH) found in the embryonic brain of the chick. The ratio value lower than 1, found in the adult chick brain, is a special biochemical feature which emerges at a certain time-point of development, occurring around the middle of the prehatching age.

It has long been recognized that the brain has the capacity to metabolize glucose through the pentose phosphate cycle[2–4], this pathway representing a functionally significant alternative route for glucose utilization, particularly related to lipogenesis and myelin formation. G6PDH and 6PGDH catalyze the two-stage, NADP dependent, oxidation of glucose-6-phosphate, thereby constituting the direct oxidative pathway of glucose metabolism.

Our previous studies on glucose metabolism in the adult chick brain[5,6] have shown that the G6PDH/6PGDH ratio is < 1, the low activity of G6PDH being responsible for this unusual feature of the oxidative moiety of the hexose monophosphate shunt. The aim of the present work is to study the patterns of G6PDH and 6PGDH activities in the chick embryo during brain development in order to establish whether G6PDH/6PGDH is < 1 in embryonic as in adult brain.

Materials and methods. Embryonated eggs were purchased from a local hatchery and incubated in a conventional cabinet electric incubator at 38 °C in a relative humidity of 50%. A number of eggs were opened on the 6th day (the tissue from earlier embryos was too small) and on every