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].

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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

day thereafter until the 21st day. Embryonic brains were then removed and freed from all other structures, such as optic cups. They were then rinsed in cold physiological saline, blotted with a filter paper, weighed and homogenized in 2 vol. of 0.1 M TRIS/HCl buffer adjusted to pH 7.6, containing 15% glycerol, 0.005 M EDTA and 0.001 M β-mercaptoethanol. A teflon homogenizer was used and the tissue processed within 30 sec; the homogenate was centrifuged at 96,500 × g in a SW 50 rotor with a Spinco Beckman L-50 centrifuge. Both G6PDH and 6PGDH were assayed in the clear supernatant. The enzyme determinations were carried out as previously described[6], using an Eppendorf recording photometer equipped with a thermoregulated cell holder. All the enzyme assays were carried out at 30 °C under linear kinetic conditions. Total proteins were determined by the biuret method according to Beisenhertz et al.[7].

Results and discussion. The developmental patterns of chick brain G6PDH and 6PGDH as a function of prehatching age, are reported in figure 1. The level of G6PDH activity shows an interesting behavior during development; in the early stages of incubation the enzymatic activity shows relatively high levels which are maintained until the 10th day of development; a marked decline in G6PDH activity follows through the remainder of the incubation period. The general developmental profile is similar to those obtained by other authors[8-10]. A different pattern is exhibited by G6PDH, whose values, except for minimal fluctuations, do not change during early or late development. The results of our investigation allow us to draw the profile of the G6PDH/6PGDH ratio in the developing chick brain; as can be seen in figure 2, the ratio is higher than 1 in the first 10 days of incubation; from the 10th day on, the ratio is lower than 1 and remains so until hatching. The low values of prehatching stages are also maintained through the posthatching and adult age. Our data moreover show that the G6PDH/6PGDH ratio drops below 1 owing to the marked loss of catalytic efficiency of G6PDH. The presence of endogenous inhibitors which might influence the activity of G6PDH has been excluded by showing that admixtures of enzyme preparations from 6- and 20-day-old embryos gave the expected sum of the 2 activities.

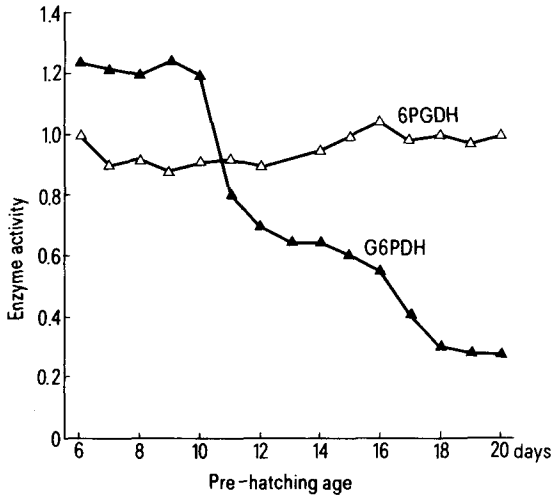

Figure 1. Developmental patterns of G6PDH and 6PGDH in chick brain. Enzymatic activities are expressed as μmoles of NADP reduced/mg protein/h. Each value is a mean of 4 or more experiments. SE never exceeded 5% of the mean.

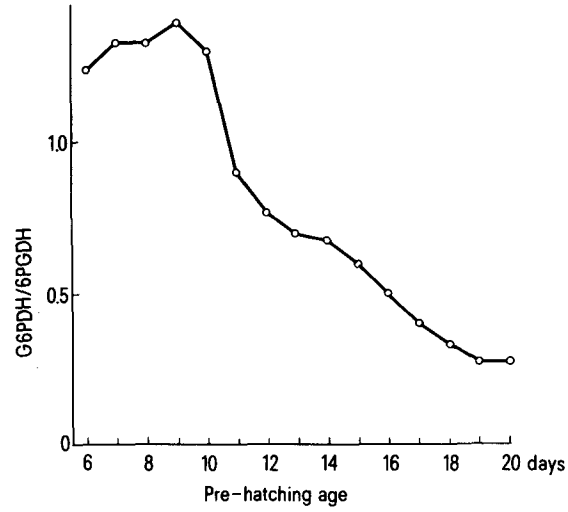

Figure 2. G6PDH/6PGDH ratio in chick brain during development.

The developmental behavior of G6PDH and of 6PGDH in the brain indicates that the hexose monophosphate shunt is distributed differently in the course of organogenesis and suggests the existence of a regulatory mechanism exerted on the 1st enzyme of the metabolic pathway. The physiological significance of this enzymatic regulation during organogenesis is given by the variable requirements of $NADPH_2$ necessary for lipogenesis and pentoses essential for nucleic acids synthesis. With reference to this, the maximal catalytic efficiency of chick brain G6PDH in the first days of incubation shows that the hexose monophosphate shunt functions more in the early stages of differentiation than during the later phases of embryonic life. Moreover, the same results indicate that the ratio G6PDH/6PGDH represents an adult biochemical characteristic which does not occur in the fertilized ovum; it emerges during the embryonic life at a developmental time-point which may represent a crucial moment of biochemical differentiation.

It is well known that during ontogenesis the morphological changes, involving variations in metabolic requirements, are often accompanied by equally specific changes in enzyme structure and properties[11-13]. In this connection, experiments are being undertaken on the physico-chemical properties of the pre-mature and mature G6PDH molecule to establish whether developmental changes in the molecular structure of the enzyme are responsible for the observed changes in catalytic efficiency.

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