HT and PEA are oxidized by either type of MAO has never been reported.

The differentiation observed in MAO inhibition by deprenyl was not so marked as that by clorgyline: neither plateaus nor shoulders were observed for all the substrates (figure, b). This situation is similar to that of rabbit tissues with deprenyl[12]. However, the substrate susceptibility was in the order consistent with the type A and type B classification. It seems likely that percent inhibition of MAO by 10^{-7} M deprenyl reflects the percentage of type B MAO in chick brain mitochondria, since the approximate reverse relationship in MAO inhibition between the 2 inhibitors was obtained at the concentration of 10^{-7} M.

In the present communication, we demonstrated that neither 5-HT nor PEA is the specific substrate for the 2 types of MAO with chick brain mitochondria. This result lends further support to the idea that the classification of MAO into type A and type B is of limited value[6].

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Tetrapyrrole biosynthesis from 4,5-dioxovaleric acid in *Rhodopseudomonas spheroides*

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Summary. Porphyrin biosynthesis from 4,5-dioxovaleric acid was studied in cell suspensions of *R. spheroides.* The experiments show that 4,5-dioxovaleric acid is a far precursor of porphyrins through δ amino laevulinic acid formation in a transamination reaction involving also l-alanine. It differs radically from the classical δ aminolaevulinic acid synthesis using glycine and succinyl CoA as substrates.

δ Aminolaevulinic acid (δ ALA), a classical porphyrin precursor, is synthetized from succinyl CoA and glycine through a reaction catalyzed by δ ALA synthetase. However, Tait[3] has suggested that δ ALA transamination might be involved in δ ALA synthesis, particularly in some systems in which no δ ALA synthetase has been detected. The substrate in this transamination is 4,5 dioxovaleric acid (DVA). Recently, Lohr and Friedmann[3] working with *Zea mays* leaf extracts found a novel NADH-dependent reduction of the 1-carboxyl group of α ketoglutarate yielding 4,5-dioxovalerate acid, followed by a transmission between this product and l-alanine to yield δ ALA. In addition, Neuberger and Turner[5], working with *R. spheroides,* pointed out that the transamination reaction favours δ ALA formation from DVA.

In this work we synthetise pure DVA using a new method described by Kissel and Heilmeyer, Jr[6], and we show that DVA is an earlier precursor of tetrapyrrole biosynthesis than δ ALA in *R. spheroides.*

Material and methods. 4,5-DVA was synthetized according to Kissel and Heimeyer, Jr[6]. Its purity was checked preparing the 2,4-dinitrophenyl osazone (m.p. 245–248 °C). Culture, growth and harvesting were performed according to Lascelles[7].

Cells were grown in anaerobiosis under light at 36–37 °C and incubations were carried out as indicated in the legends of the figures. After different times of incubation, the cell suspensions were centrifuged at 40,000 × g and the porphyrin content of supernatants was determined spectrophotometrically using the extinction coefficient for coproporphyrin III[8].

Results and discussion. As is shown in figure 1, a small amount of porphyrins can be synthetized without l-alanine being present. When this amino acid was added, porphyrin synthesis increased, indicating that DVA is a precursor of porphyrins through a transamination involving δ ALA. l-Glutamic acid cannot replace l-alanine as a transamination substrate. In figure 2 it is shown porphyrin formation

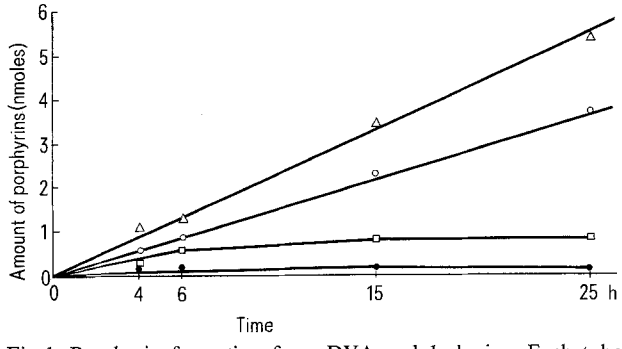

Fig. 1. Porphyrin formation from DVA and l-alanine. Each tube contained 3 mg/ml cell suspensions; 0.1 M phosphate buffer pH: 6.9; 8×10^{-3} M DVA; 8×10^{-4} M $MgSO_4$ and the following l-alanine concentrations: □, none; ○, 8×10^{-3} M; △, 1.6×10^{-2} M; ●, blank without DVA and 1.6×10^{-2} M l-alanine. Incubations were carried out in anaerobiosis under light at 36–37 °C.

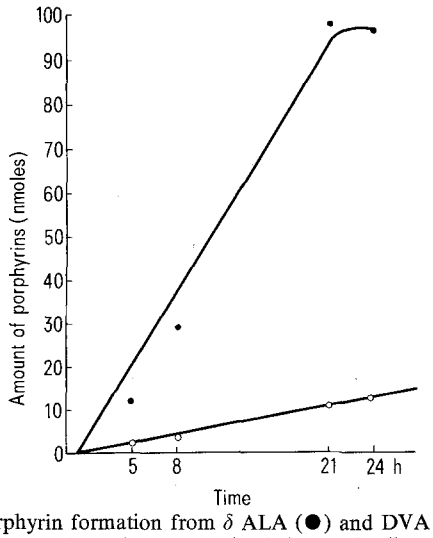

Fig. 2. Porphyrin formation from δ ALA (●) and DVA (○) plus alanine. The reaction mixture contained: 3 mg/ml cell suspensions; 0.1 M phosphate buffer pH: 6.9; 10^{-3} M $MgSO_4$ and 10^{-2} M δ ALA or 10^{-2} M DVA plus 10^{-2} M l-alanine. Incubations were performed as indicated in figure 1.

from a) δ ALA and b) DVA plus l-alanine. As expected, δ ALA gives rise to a greater amount of porphyrins as compared with DVA plus alanine.

In the supernatants, after sonications of cell suspensions, we were able to detect activity of l-alanine-4,5-dioxovalerate amino transferase (transaminase) according to Gibson, Neuberger and Tait[9]. The reaction is linear up to 16 min using this crude extract.

In a previous paper[10], working with the same bacteria, we reported our studies on the incorporation of labelled δ ALA into the purine ring of nucleotides. Our results are not in agreement with a direct incorporation of δ ALA into the ring through transamination of this metabolite to DVA. Instead we suggested that DVA transamination with l-alanine to yield δ ALA would be favoured.

From the results reported here, DVA is a far precursor of porphyrins in cell suspensions of *R. spheroides;* δ ALA is formed through a transamination reaction between l-alanine and DVA. Beale, Gouch and Granick[11] and Lohr and Friedmann[4], working with plants, found a different route of δ ALA synthesis that is completely different from the classical pathway involving glycine and succinyl CoA. Our findings from *R. spheroides* are consistent with this new metabolic pathway.

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Abbreviations: δ ALA, δ aminolaevulinate acid; DVA, 4,5-dioxovaleric acid.

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Brain microvessel hexokinase: Kinetic properties

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Summary. Kinetic differences between brain capillary and parenchymal hexokinase in the presence of glucose, ATP, fructose, potassium, sodium and different pH were established. Parenchymal hexokinase is more susceptible to glucose inhibition, can tolerate greater variations in the ATP concentration, is inhibited by increasing concentrations of fructose and potassium, and showed greater activity on the lower pH values. The data suggest that in brain parenchyma and endothelial cells of brain microvessels, there are 2 different enzymes with regard to the kinetic properties.

Brain microvessels are unique with respect to their barrier mechanism; selective transport of substrates from the blood to brain has been attributed to the specific biochemical organization of the endothelial cells of cerebral capillaries. In an attempt to establish some of the metabolic properties of brain microvessels at the cellular level, we have begun to study capillaries isolated from the brain[1,2]. Recently, we have proposed the possible role of hexokinase (HK) in carrier-mediated glucose transport from the blood into the brain parenchyma[3].

In the hexokinase (ATP: D-glucose-6-phosphotransferase, EC 2.7.1.1) reaction, glucose-6-phosphate (glucose-6-P) is formed from glucose and adenosine triphosphate (ATP). Glucose-6-P can be directed towards a) glycogen synthesis, b) pentose phosphate pathway, and/or c) glycolysis. The glycolytic pathway is the main energy source in a number of tissues, particularly in the brain, where HK acts not only at the point of 'distribution' of glucose-6-P into different metabolic pathways but is regulatory enzyme in glycolytic flux as well[4].

In the previous study, noticeable HK activity was found in isolated brain microvessels in comparison with the brain parenchyma; it was striking to find that the activity of phosphofructokinase (EC 2.7.1.11; PFK), the key enzyme of glycolysis[4], was low in brain microvessels when compared with the HK activity[5]. Hence, there is great difference in the HK/PFK ratios between brain microvessels and parenchyma indicative for possible distinct roles of HK in these 2 compartments. Therefore, we made an attempt to establish kinetic differences between 2 HK's (capillary and parenchymal) in the presence of glucose, ATP, fructose, potassium, sodium and different pH.