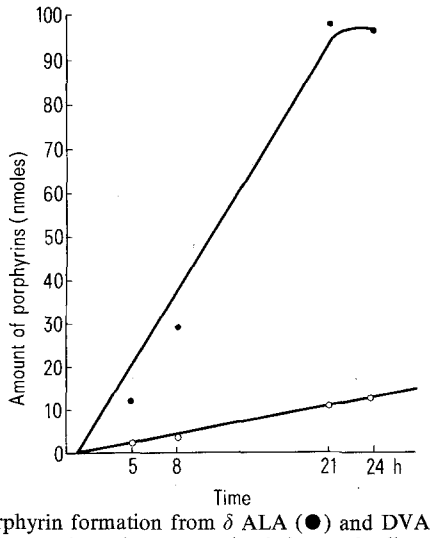

Fig. 2. Porphyrin formation from δ ALA (●) and DVA (○) plus alanine. The reaction mixture contained: 3 mg/ml cell suspensions; 0.1 M phosphate buffer pH: 6.9; 10^{-3} M $MgSO_4$ and 10^{-2} M δ ALA or 10^{-2} M DVA plus 10^{-2} M l-alanine. Incubations were performed as indicated in figure 1.

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.

Materials and methods. Adult male Wistar rats were sacrified by decapitation, skulls were quickly removed, and forebrain homogenized in 5 vol. of the ice-cold homogenizing medium as described elsewhere[5]. The procedure of isolation of the pure microvessel fraction was essentially the same as described by Mršulja et al.[1], as modified for the enzyme preparation from microvessels and parenchyma by Djuričić and Mršulja[5]. Hexokinase activity was determined following the appearance of NADPH at 340 nm in Beckman DB-G spectrophotometer with thermospaced cuvette holder (37 °C). The assay mixture used for the determination of the baseline activity and for the effect of Na^+ and K^+ concentrations was composed as follows (final concentrations): Imidazole-Cl buffer, 50 mM, pH 7.1; $MgCl_2$, 10 mM; dithiotrietol, 0.1 mM; $NADP^+$, 0.2 mM; glucose, 0.5 mM; ATP, 0.5 mM; glucose-6-P-dehydrogenase (EC 1.1.1.49), 0.2 U/ml. When the effects of glucose concentrations were studied, ATP concentration was 4 mM; in the study of the effect of ATP, glucose concentration was 4 mM and imidazole buffer was replaced with the HEPES buffer, 50 mM, pH 7.1. For the fructose studies, ATP concentration in the assay mixture was 4 mM and phosphoglucoisomerase (EC 5.1.3.9), 0.2 U/ml was added. In the study of the effect of the pH changes, imidazol-Cl buffer of indicated pH values were used. Reaction was initiated by the sample adding, and reaction velocities were linear with respect to the time and protein concentrations. Protein concentrations were estimated according to Lowry et al.[6], with bovine serum albumin as the standard. Michaelis-Menten constant were obtained from the double reciprocal plots of Lineweaver-Burk in a non-inhibitory range of the substrate concentrations.

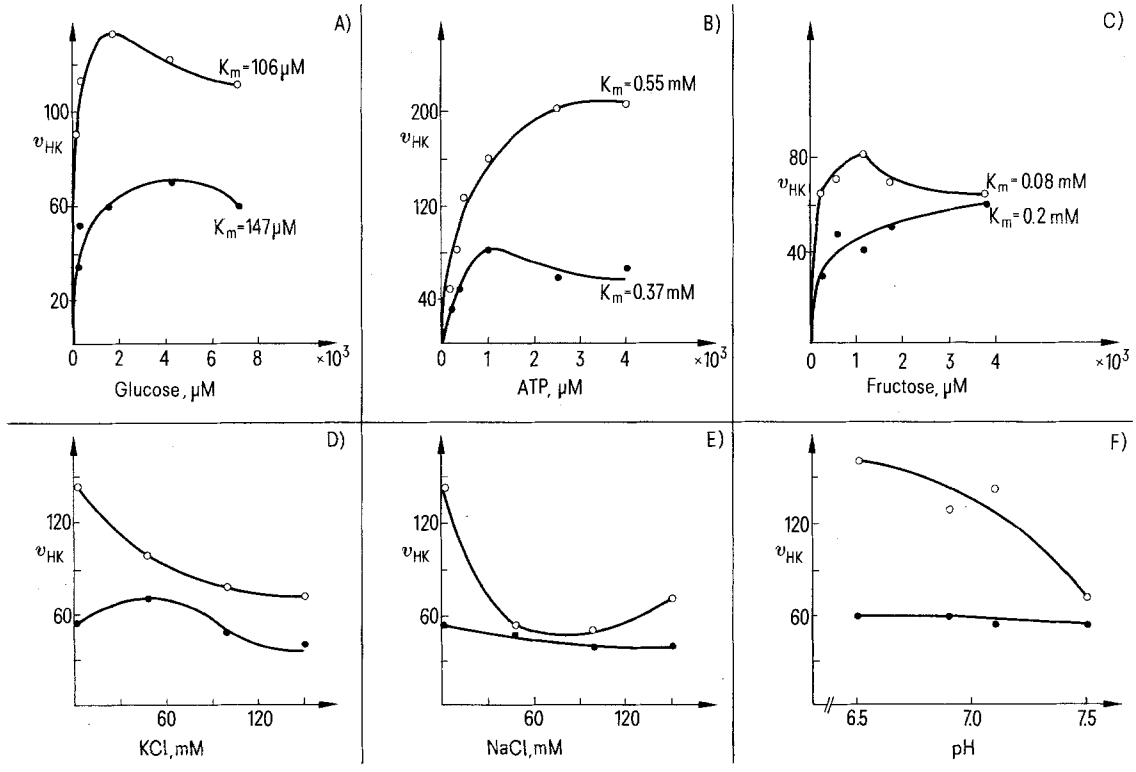
Results and discussion. The HK activity was 147±6 nmoles of glucose/mg protein/min (mU/mg protein) and 56±2 mU/mg protein in brain parenchyma and microvessels, respectively.

Figure, A. When glucose concentration was varied up to 8 mM, parenchymal HK responded with high increase in activity to about 2 mM of glucose being inhibited after that by the substrate. In microvessels, the inhibition was less pronounced and the increase in activity was observed up to 4 mM of substrate. Michaelis-Menten constant (K_m) for 2 HKs were 0.106 mM and 0.147 mM for the parenchymal and microvessel enzyme, respectively. Hence, capillary enzymes can tolerate relatively great changes in glucose concentration (approximately from 0.5 to 4 mM) without significant changes in activity; concentration range is more narrow for the parenchymal HK.

Figure, B. Variations in ATP concentrations up to 4 mM resulted in slight inhibition of microvessels HK, while apparently there was no inhibition of parenchymal enzyme; respective K_m's for this substrate were 0.366 mM and 0.554 mM. These findings may be related to the lower enzyme metabolism of the endothelial cells in comparison with the parenchymal cells, and consequent lower intracellular ATP concentration in capillaries[5].

Figure, C. When fructose was used instead of glucose as the substrate, in parenchyma the activity was about half of that with glucose exhibiting inhibitory effects with concentrations greater than 1 mM; K_m-value was 0.081 mM. In brain capillaries, with the increasing concentrations of fructose, HK activity reached the activity found to be maximal for the glucose; K_m-value was 0.196 mM. In general, curves for fructose followed those for glucose, except that the parenchymal enzyme is more substrate specific.

Figure, D and E. Parenchymal enzyme activity decreased with the increase in potassium concentration. Microvessel HK responded biphasically: up to 50 mM potassium stimulated HK, but further increase inhibited it. Increase in sodium concentrations caused also biphasic respond of the parenchymal HK: up to 70 mM of sodium concentration caused inhibition of HK, while further increase in sodium



Kinetic properties of hexokinase (HK) in rat forebrain microvessels (●) and parenchyma (○) in the presence of different substrates and ions. Each point represents the mean value of 6 measurements done in duplicates. For details see text.

concentration was followed by the partial de-inhibition of the enzyme. In capillaries response was slow; there was almost linear inhibition of the enzyme with increasing concentrations of sodium. It is clear that microvessel HK is less sensitive to the ionic changes, while parenchymal HK response favors demands for enhanced energy production after neuronal firing when intracellular potassium is lowered and sodium increased.

Figure, F. The further evidence that microvessel HK is less sensitive to the ionic changes, came from the fact that this enzyme was insensitive for the changes in pH, while parenchymal HK activity decreased with the increase of pH from 6.5 to 7.5. One can speculate that this could be related to the some trigger mechanism for the glycolysis, particularly in the case of lactate accumulation. In hepatic cells, lactate serves as a signal for enhanced glycolysis[7].

From our data, microvessel and parenchymal hexokinase are clearly distinguished enzymes, at least kinetically. Our attempt to reveal isoenzyme differences of 2 HK's using acrylamide disc electrophoresis was not successful. However, one must be aware of the complex nature of brain HK, for it is known that it is mainly particle-bound enzyme[8,9]; in our preparation about 60% of the total HK activity was particle-bound. Hence, HK may play some additional roles besides generating G-6-P for glycolytic pathways[10]. In parenchyma, its main function is to increase energy production when the cell demands it, and its kinetic properties are in agreement with this function. In microvessels the function of HK seems to be different. Endothelial cell itself has not great energy demands[3,5]. Hexokinase, therefore, may be incorporated in the glucose transport system through endothelial cell along with glucose-6-Pase[3]; excess of G-6-P not metabolized by endothelial cell itself may be hydrolized via glucose-6-Pase reaction and glucose released in juxtaposed glial cell. The nature of glucose carrier through membranes of endothelial cells remains to be elucidated, but there is evidence that transport of glucose into capillaries is saturable with K_m-values very close to microvessel hexokinase K_m (figure, A)[1]. However, whether different kinetic properties of the enzyme are related to the specific functions of the cell remains to be established. This could be of particular interest for the endothelial cell, most likely to be a site of blood-brain barrier.

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Inheritance of four characters in *Dolichos lablab* L. (Leguminosae)

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Summary. Trigenic ratios have been reported for the first time for the following characters: Habit of the plant, inflorescence type, pod form and pod colour. The first two characters show the presence of 3 common genes and the latter ones are independent.

Dolichos lablab L. (*Lablab niger* Medic., Syn. *Lablab purpureum* sweet), a climbing annual, is popularly known as field-bean. However, systematic crop improvement has not been done. Hence, a program on genetic improvement of this crop through germplasm collection, evaluation and hybridisation has been taken up by the Department of Agricultural Botany, University of Agricultural Sciences, Hebbal, Bangalore.

The 2 varieties, *Hebbal Avare-1* and *Ginnu,* were crossed and F_1, F_2 generations were studied. *Hebbal Avare-1* is a variety released at the University of Agricultural Sciences, and *Ginnu* is a locally cultivated variety around Bangalore. *Hebbal Avare-1* is erect in plant habit with the racemose type of inflorescence (florets borne on the erect floral axis), the pod is flat and white in colour. *Ginnu* is twining in habit with the flowers borne in clusters at the nodes (in the axil of every leaf), pod inflated and green in colour.

The data on parents, F_1 and F_2 are given in table 1. The F_1

Table 1. Characters of parents, F_1 and F_2 segregation of the cross *Hebbal Avare-1* (P_1) × *Ginnu* (P_2)

Characters involved in crosses		F_1		F_2	Ratio	χ^2	p
Habit	P_2 twining P_1 erect	Twining	dom. rec.	872 345	45:19	1.04	0.4–0.3
Inflorescence	P_2 axillary cluster P_1 terminal long-stalked	Cluster	dom. rec.	868 349	45:19	0.59	0.5–0.4
Pod form	P_1 flat P_2 inflated	Flat	dom. rec.	1068 149	57:7	2.13	0.2–0.1
Pod colour	P_2 green P_1 white	Green	dom. rec.	1195 22	63:1	0.47	0.5–0.4