ASSESSMENT OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE ROLE IN ANAEMIA OF CHRONIC RENAL FAILURE

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By

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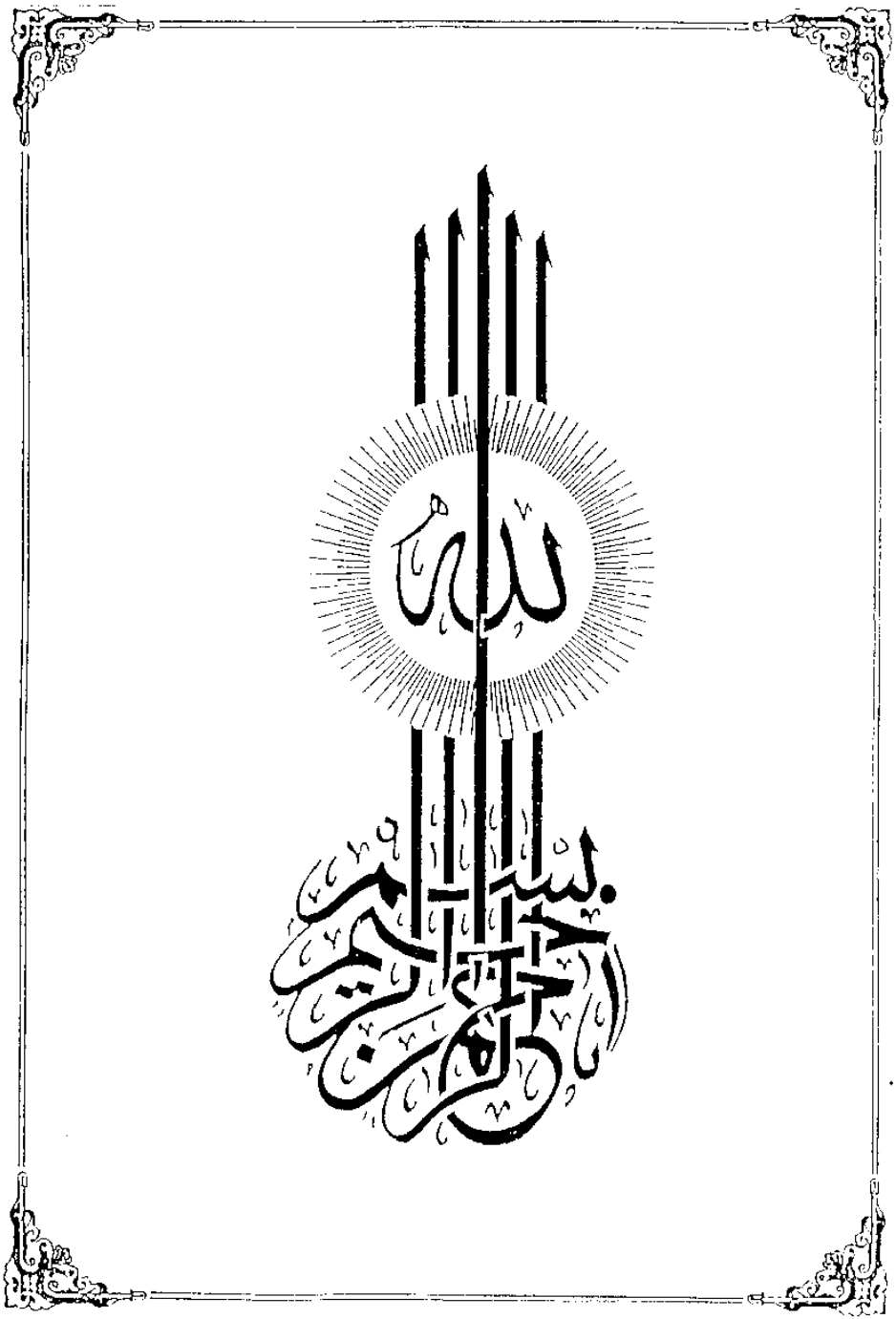

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LIST OF ABBREVIATIONS

ADP	:	Adenosine di-phosphate
ATP	:	Adenosinetri-phosphate
CFU-E	:	Colony forming units-erythroid
DPG	:	Diphosphoglycerate
EMP	:	Embden-Meyerhof pathway.
FDP	:	Fructose diphosphate
G.F.R.	:	Glomerular filteration rate.
G3P	:	Glyceraldehyde 3-phosphate
G6P	:	Glucose 6-phosphate
G6PD	:	Glucose 6 phosphate dehydrogenase
G-SH	:	reduced glutathione
GSSG	:	Oxidized form of glutathione
HB	:	Haemoglobin
HMP	:	Hexose monophosphate shunt
MDA	:	Malonyldialdehyde
NADH	:	Reduced nicotinamide adenine dinuleotide
NADPH	:	Reduced nicotinamide adenine dinulceotide phosphate.
PfK	:	Phospho-fructo-kinase
6PG	:	6-Phosphogluconolactone
PPP	:	Pentose phosphate Pathway
PTH	:	Parathyroid hormone
Ru-5P	:	Ribulose 5-Phosphate

INTRODUCTION & AIM OF THE WORK

INTRODUCTION

AND AIM OF THE WORK

Among many symptoms of chronic renal failure, anaemia stands as a hallmark and is one of the diagnostic clues to the clinicians. It is one of the early laboratory abnormalities in chronic renal failure and is usually normocytic normochromic. (Fried, 1971)

Decreased red cell life span is a constant feature in all patients with renal failure. (Anagnostou and Kurtzman, 1986)

Glucose - 6 - phosphate dehydrogenase is the enzyme that catalizes the first step in Hexose - monophosphate - shunt (H.M.P-shunt) in which reduced nicotinamide adenine dinucleotide phosphate is generated. NADPH maintains glutathione in its reduced form and this protects haemoglobin and red cell membrane from excess oxidant stress.(Grimes, 1980).

Glucose - 6 - phosphate dehydrogenase deficiency is a well known cause of enzyme deficiency induced hemolytic anaemia.(Murphy, 1985).

The aim of our work is to assess the role of glucose - 6 - phosphate dehydrogenase enzyme in anaemia in chronic renal failure patients under both conservative and haemodialysis treatment.

REVIEW OF LITERATURE

CHRONIC RENAL FAILURE :

Definition:-

Chronic renal failure is a syndrome which develops as a consequence of persisting and progressive loss of nephron function and is due to the resultant impairement of renal excretory, metabolic, endocrinal and homeostatic functions (Davison , 1981).

Diminished renal reserve is the 1st stage renal failure where plasma biochemistry is normal and the abnormality in renal function is detected as a decrease in glomerular filteration rate (G.F.R). Diminished renal reserve becomes early failure at G.F.R. about 30 ml per minute, late renal failure at 10 ml per minute and end-stage renal failure at 5 ml per minute. Uraemic symptoms which appear in early chronic renal failure evolve into the full uraemic syndrome during late and end-stage failure (Oliver, 1983).

Red cell metabolism in normal and uraemic persons:

Normal Red Cell Metabolism:

As the red cell emerges from the bone marrow, it loses its nucleus, ribosomes and mitochondria. Therefore, all capabilities for cell division, protein synthesis and oxidative phosphorylation are lost. However, red cells are metabolically quite active utilizing some 1.5 to 2.2 mmol glucose / litre / hour (Bell, 1980).

Compared to other cells, the red cell has a rather simple scheme of intermediary metabolism. While other cells can utilize glucose, galactose, fructose and mannose. Glucose is virtually the only fuel utilized by red cells (Bonsignore, et al.1963).

Glucose degradation within the red cell serves three functions:

1. Maintenance of cell structure and shape,
2. Maintenance of hemoglobin in the reduced form, and
3. Modulation of the oxygen affinity of hemoglobin (Grimes, 1980).

Glucose readily enters the red cell by facilitated diffusion and is independent on insulin (Smith, et al.1983). Glucose is then converted to glucose - 6 - phosphate.

There are two major pathways available for glucose - 6 - phosphate, glycolysis and the pentose - phosphate shunt.

About 80 - 90 % of this intermediate is converted to lactate by means of the glyclytic (or Embden - Meyerhof) pathway. The primary role of the glycolytic pathway is to produce high energy phosphate adenosine triphosphate (ATP) and NADH. The intracellular mediator of hemoglobin function, 2,3 diphosphoglycerate (2,3 DPG) is synthetized in a two enzyme side reaction. This is Known as the Rapoport - Leubering Shuttle (Smith, et al.1983).

10 % of glucose - 6 - phosphate metabolism undergo oxidation by means of the hexose monophosphate shunt (HMP). The priciple function of this pathway is to produce reducing energy equivelants in the form of NADPH and thereby maintain glutathione in the reduced state (Grimes, 1980).

Therefore, two resrvoir of energy exist in the red cells. One is of reducing energy, the other consists of high energy phosphates in the form of ATP and DPG. (Mayes,1983).

Reducing power for reduction of methemoglobin is supplied in the form of NADH is present by glycolysis.Another methemoglobin reductase employing NADPH is present in the erythrocyte, but it acts only in the presence of a dye such as methylene blue and its physiological role if any is unclear (Smith, et al.1983).

Reducing power for counteracting oxidant stress on the membrane and globin part of hemoglobin comes mainly from the NADPH produced by the pentose phosphate shunt acting through glutathione system (Bell,1980).

Glutathione provides reducing activity by serving as a sacrificial reductant. The resulting oxidized glutathione (GssG) is catalytically reconverted to G-SH by the action of glutathione reductase using NADPH as a cofactor (Harper,et al.1977).
An overview of erythrocyte glucose metabolism is provided in the figure (1A & B).

Hexose Monophospjate Shunt (HMP-Shunt):-

The sequence of reactions of the shunt pathway may be divided into two phases. In the first phase glucose - 6 - phosphate undergoes dehydrogenation and decarboxylation to give the pentose , ribulose - 5 - phosphate. In the second phase ribulose - 5 - phosphate is converted back to glucose - 6 - phosphate by a series of reactions involving mainly 2 enzymes, transketolase and transaldolase (Mayes, 1983).

Glucose - 6 - phosphate dehydrogenase is the first enzyme of pentose phosphate pathway (PPP or HMP), and catalyses the conversion of glucose - 6 - phosphate to 6-phosphogluconolactone with the concomitant NADPH production.

It is generally agreed that the activity of HMP-shunt is controlled at the G6PD step by the availability of $NAPD^+$, a fact first established using rat liver slices by Cahill,et al.(1958). The pathway can be stimulated by the presence of methylene blue and the maximum rate of stimulation achieved is in constant ratio to the maximum velocity of G6PD reaction measured in dilute hemolysates and using different mutants of G6PD with subnormal activity. This suggests that G6PD activity can exert some control on the rate of HMP-shunt. G6PD exists in a large number ofmutant forms, two of them are very common and all axcept one or two have an activity which is less than that of normal form. Normal G6PD is designated as B and consists of tetramer of four apperently identical subunits each with a molecular weight of about 25,000. The monomer is inactive but the dimere and tetramer are in equilibrium and the dimer is probably the active form in vivo (Grimes,1980).

G6PD is the best studied of human polymorphic enzymes as it exists in the form of over 200 variants which differs from one another in their activity, electrphoretic mobility, stability and specificity towards different substrates (Warsy, 1987).

Transketolase is another enzyme which is involved in the steps of HMP-shunt, it transferes 2 carbon moity from xylulose - 5 - phosphate to ribose - 5 - phosphate. This enzyme recycles glucose into HMP-shunt.

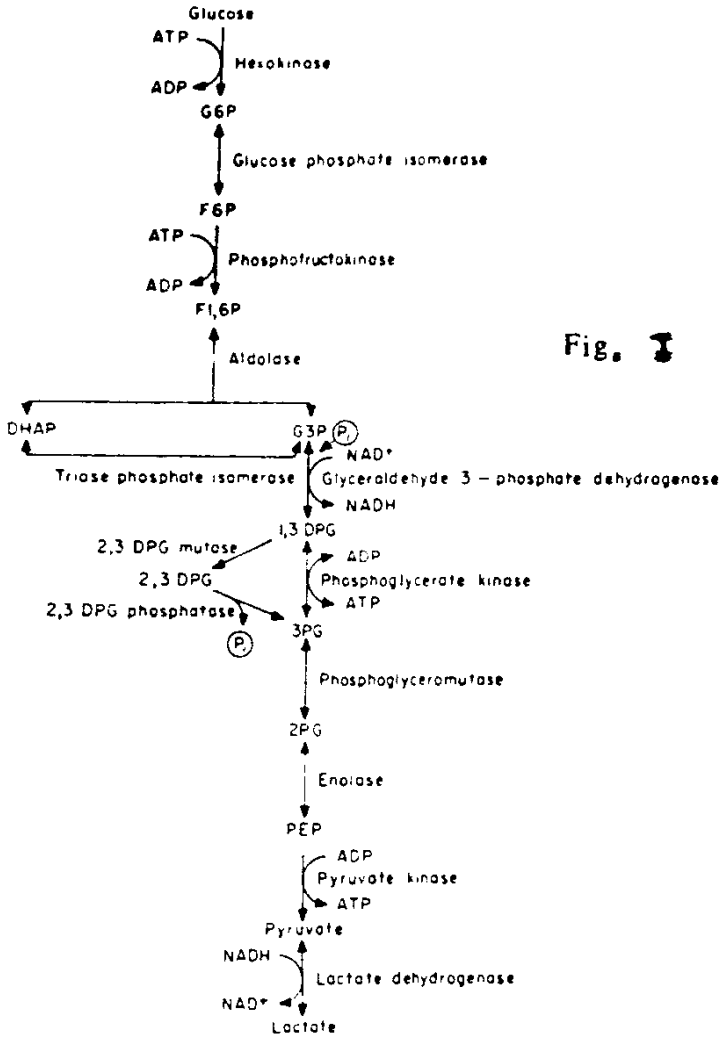
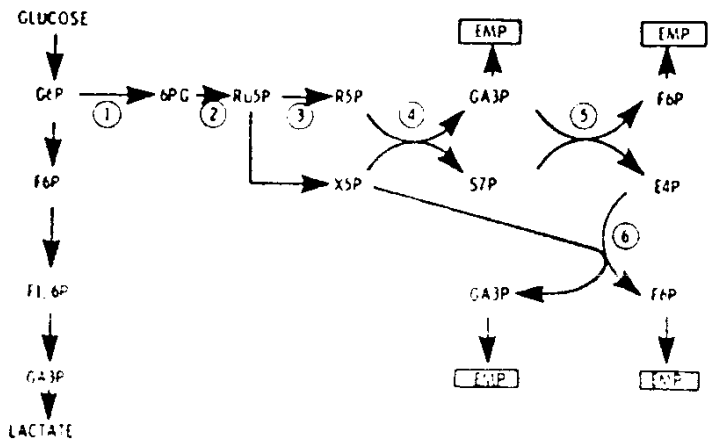


Fig. 1

A – The Embden-Meyerhof pathway (EMP) in human red cells



B – The hexose monophosphate pathway (HMP). The abbreviated EMP shows the two intermediates (F6P and GA3P) common to both pathways ① glucose 6-phosphate dehydrogenase, ② 6-phosphogluconate dehydrogenase; ③ isomerase; ④ and ⑤ transketolase, ⑥ transaldolase

Measurement of HMP-Shunt Activity :-

The liberation of Co_2 during the conversion of 6PG into Ru-5P appears to be the only site in mature red cell for metabolic production of Co_2. For this reason, incubation of red cells with $^{14}C_1$ glucose, release $^{14}Co_2$ at a rate which is a direct measure of HMP- activity. This technique has been used, with many modifications, since 1960 (Grimes,1980). The results obtained for HMP-activity are expressed either as a percentage of overall glucose consumption or as an amount of glucose passing through HMP-shunt in unit time.

Davidson and Tanaka (1973), developed another technique for continous monitoring of $^{14}Co_2$ from incubates of cells containing ^{14}-glucose. This method has the advantage of recording changes in HMP activity directly following the application of a stimulus, thus studying factors which affect HMP-activity.

The principle of using $^{14}C_1$ glucose to assess HMP-activity is valid only provided that recycling through the pathway does not occur, so, this will tendto under-estimate HMP-activity (Davidson and Tanaka, 1969), and it appears that red cells are incubated with $^{14}C_2$ labelled Co_2 is five to one (Grimes, 1980). So, measurement of both glucose 1-C^{14} and glucose 2-C^{14} oxidation to Co_2 will be more accurate (Davidson and Tanaka, 1972).

Factors Affecting HMP-Activity :-

Activity of both EMP and HMP pathways increases with a rise of pH, but HMP-shunt is considerably less pH-sensitive than EMP, thus, at pH 7.0 HMP activity is approximately 8 % of EMP activity, but at pH 7.7, this value becomes nearer 4 % (Grimes, 1980).