Biochemical profile of erythrocyte membrane of jaundiced neonates

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Studies in newborn humans have demonstrated alteration in the lipid, phospholipid and cholesterol content when compared with age-matched control. Membrane bound (Na⁺+K⁺)ATPase activity is found to be significantly increased in jaundiced neonates. Alteration in membrane permeability characteristics in jaundiced neonates causes severe microenvironmental changes in red blood cell profile.

Jaundice is observed during the first week of life in approximately 60% of term infants and 80% of preterm infants¹. The unconjugated bilirubin has been found to be neurotoxic². When the serum bilirubin rises above a threshold level, the unconjugated bilirubin crosses the blood brain barrier resulting in hyperbilirubinemic encephalopathy or kernicterus. A dose response relationship between maximal total serum bilirubin concentration and odds of handicap has also been observed³. Toxicity is further potentiated by low birth weight, perinatal asphyxia, acidosis, hyperthermia and hypoglycemia⁴. It has been reported from this laboratory that reduced glutathione (GSH) level is depleted from R.B.C. of jaundiced neonates⁵. This observation rationalises the thinking of change in permeability characteristics of erythrocyte membrane. The functioning of erythrocyte membrane constituted by lipid, lipoproteins and cholesterol in the jaundiced neonates is, therefore, expected to change which may result in altered permeability. Membrane lipids vary both in type and in their proportional distribution. The organism, the cell type and even the particular organelle membrane determine the specific difference in lipid composition⁶. The lipids are responsible for approximately one half of the mass of R.B.C. membranes. Phospholipid and cholesterol account for the vast majority of these lipids and are both present in almost equal amount in normal cell membrane. The large mass of fatty acyl groups in these membrane phospholipids probably influence the physical and permeability characteristic of the membrane to a significant extent and changes in their composition may be expected to change in their characteristics⁷. Some lipids have been implicated in the transport of ions, in bacteriogenesis⁸, in the receptor properties and in the enzyme activities of the membrane⁹. Although many theories have been proposed for the anatomic localization of the lipids within erythrocyte membrane but their changes, if any, in jaundiced neonates is still unknown. The changes in membrane lipid profile may affect the activity of membrane bound (Na⁺+K⁺)ATPase activity. Inhibition or activation of this enzyme which mediates active transport across the membrane has been reported to affect cell replication¹⁰. A recent study also suggests that porphyrin induced photosensitization may contribute to cytotoxicity through inhibition of (Na⁺+K⁺)ATPase activity. Cell membrane bound (Na⁺+K⁺)ATPase represents the machinery that subserves active transport of Na⁺+K⁺¹¹. It has now been recognized that erythrocyte membrane (Na⁺+K⁺)ATPase could be used as a model for the study of generalised membrane defects in the cation transport¹². Since there is no protein synthesis in R.B.C. membrane¹³ the question of possibilities of absolute decrease or increase in enzyme activity in these cells does not arise. The lowering or elevation of enzyme activity may be brought about from altered membrane microenvironment which in turn may modify the enzyme molecule thereby modifying its characteristics in jaundiced neonates. The present study is therefore undertaken to evaluate i) the lipid

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profile of erythrocyte membrane, and ii) the activity of membrane bound Na+/K+-ATPase.

The study include 60 neonates both full term (35) and preterm (25) of which 39 were males and 21 females (age between 1 and 30 days). They were admitted to the nursery of the Institute of Child Health, Calcutta. The birth weights of both the control and study group were in the range of (1800-2500) g. The mean ± SD of total serum bilirubin level in the study group was (20 ± 6.8) mg/dl at the initial examination. The patients has no history of blood transfusion or phototherapy at the time of investigation. Their mothers had not taken any medication containing exogenic bile acids. As control 45 age matched non-jaundiced neonates (25 preterm and 20 full term) without hepatic and intestinal diseases were examined. Whole blood was collected from the patients and control group by vein puncture. One part of whole blood was collected in a vial containing heparin and the other part of it was allowed to clot in a separate vial.

Serum bilirubin with its differential fraction was estimated by the method of Evelyn and Malloy. Serum cholesterol was estimated by the method of Zak et al. Total protein was estimated by the method of Lowry et al. The concentration of serum Na+ and K+ were measured by Flame photometry. From the heparin vial plasma was separated by centrifugation at 2500 g for 10 min at 0°C. The red supernatant was discarded. The process of washing with distilled water was repeated till a colourless supernatant was obtained. The colourless supernatant was discarded and the precipitate which constituted erythrocyte membrane was suspended in isotonic saline (0°-4°C) for further investigation. Total lipid of erythrocyte membrane was extracted by the method of Rose and Oklander using chloroform, isopropanol (7:1:v:v) mixture. The extract was sampled for the gravimetric estimation of total lipid. Total phospholipid phosphorus of erythrocyte membrane was estimated according to the method of Rose and Oklander after digestion with perchloric acid. The liberated phosphate was measured by the method of Fiske and Subbarow. Micrograms of lipid phosphorus was multiplied with 0.025 to give mg of phospholipids. Individual phospholipids were separated by one dimensional TLC using solvent system of chloroform: methanol:acetic acid: water :: 25:15:4:2 (by vol). The individual phospholipids were identified with reference standards and were scrapped from the plate with a sharp needle and used for phospholipid phosphorus estimation. ATPase activity was measured according to Swanson et al. with minor modification for the use in R.B.C. The assay system contained 20 mM Tris-HCl (pH 7.2), 3 mM Tris-ATP, 3 mM Mg2+ with or without 30 mM K+ and 100 mM Na+ in a total volume of 1 ml. For the assay of ouabain insensitive ATPase the system also contained ouabain. Ouabain sensitivity is measured by subtracting ouabain sensitive ATPase (with ouabain) from total ATPase (without ouabain). The enzyme activity is expressed as µmole of phosphate liberated per mg of protein per hour and (Na+ + K+ + Mg2+)ATPase activity was calculated by subtracting Mg2+ ATPase activity from total i.e., (Na+ + K+ + Mg2+)ATPase activity.

<table>
<thead>
<tr>
<th>Study group</th>
<th>Total bilirubin (serum) mg/dl</th>
<th>Unconjugated bilirubin (serum) mg/dl</th>
<th>Total lipid mg/100 mg of total lipid</th>
<th>Total cholesterol (C) mg/100 mg of total lipid</th>
<th>Total phospholipid (T.P.L.) mg/100 mg of total lipid</th>
<th>Cholesterol phospholipid ratio x100 C/TPL x100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre term</td>
<td>1.5±0.5</td>
<td>0.5±0.2</td>
<td>85±1.2</td>
<td>38±2.5</td>
<td>58±2.6</td>
<td>68±2.6</td>
</tr>
<tr>
<td>Age 1-30 days</td>
<td>0.5±0.2</td>
<td>Undetectable</td>
<td>88±3.2</td>
<td>40±3.6</td>
<td>59±1.8</td>
<td>69±1.6</td>
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<td>Patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre term</td>
<td>21.5±6.8*</td>
<td>19.9±5.5*</td>
<td>64.3±2.05*</td>
<td>31.9±2.3*</td>
<td>53.2±2.1*</td>
<td>61.8±4.5*</td>
</tr>
<tr>
<td>Age 1-30 days</td>
<td>20.5±6.5*</td>
<td>19.5±5.8*</td>
<td>68.3±2.05*</td>
<td>32.9±3.9*</td>
<td>52.2±2.0*</td>
<td>62.5±4*</td>
</tr>
</tbody>
</table>

* Figures in the parentheses indicate number of cases in each group.  
* P < 0.005
Table 2—Individual phospholipid content and (Na⁺/K⁺)ATPase activity of erythrocyte membrane of jaundiced neonates

<table>
<thead>
<tr>
<th>Study group</th>
<th>(Na⁺/K⁺)ATPase</th>
<th>Total bilirubin (Serum) mg/dl</th>
<th>T.P.L. mg/100 mg lipid</th>
<th>% Total phospholipid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preterm (25)</td>
<td>3.04±0.22</td>
<td>1.5±0.5</td>
<td>58.5±2.6</td>
<td>22.3±0.3</td>
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<tr>
<td>Age 1-30 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Full term (20)</td>
<td>3.06±0.28</td>
<td>0.5±0.2</td>
<td>59.8±1.8</td>
<td>22.6±0.3</td>
</tr>
<tr>
<td>Patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preterm (25)</td>
<td>4.03±0.20*</td>
<td>21.5±6.8*</td>
<td>53.2±2.2</td>
<td>18.9±0.1</td>
</tr>
<tr>
<td>Age 1-30 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Full term (35)</td>
<td>4.15±0.22*</td>
<td>20.5±6.5*</td>
<td>52.2±2.0*</td>
<td>18.9±0.4*</td>
</tr>
</tbody>
</table>

*P < 0.005

P₁ = inorganic phosphorus.
P.S. = Phosphatidyl serine. P.E. = Phosphatidyl ethanolamine.

For statistical analysis, means were calculated for some continuous variables; the differences between means were analysed using the Student's 't' test. Curves and P values less than 0.001 were taken as highly significant.

The results presented in Table 1 indicate the total lipid phospholipid and cholesterol content of erythrocyte membrane of jaundiced neonates are significantly lower than that of control group. The cholesterol/phospholipid ratio is significantly lower in the patients compared with the normal neonates. No significant difference was observed between full-term and preterm neonates. The serum cholesterol content [(235±9.2) mg/dl] of jaundiced neonates is found to be significantly higher than the serum cholesterol content [(130±15.5) mg/dl] of control group. [not shown in tables].

The results presented in Table 2 indicate that phospholipid fractions are significantly lower in jaundiced neonates. The cellular (R.B.C.) K⁺ is depleted to a large extent enhancing the extracellular K⁺ to a very high level. The flux of extracellular Na⁺ in the study group, however remains almost within lower range of normal compared with non-jaundiced neonates (not shown in the tables).

Although the exact role of cholesterol in plasma membrane is not known, studies with models of biological membranes suggest that cholesterol could regulate the permeability of membranes by affecting the internal viscosity and molecular motion of lipids within the membrane [22,23]. Slightest alteration of cholesterol in the R.B.C. membrane leads to disturbances in their function [24,25]. It has been reported from this laboratory that antioxidant defence property of erythrocyte membrane are altered in jaundiced neonates [6]. The decrease in cholesterol content in erythrocyte membrane of jaundiced neonates, therefore, may be induced by the alteration in the antioxidant defence property of the erythrocyte with rise in serum unconjugated bilirubin in jaundiced neonates. Reports which are now available indicate that cholesterol/phospholipid ratio of the membrane determine its permeability characteristics and any alteration in this ratio may reflect alteration in the permeability of the erythrocyte membrane [26-30]. It is evident from Table 1 that cholesterol/phospholipid ratio is altered in jaundiced neonates. This alteration, therefore results in altering ion permeability across the erythrocyte membrane of jaundiced neonates. Of the individual phospholipids studied (Table 2) a significant decrease of phosphatidyl choline, phosphatidyl serine, phosphatidyl ethanolamine are observed. This alteration may well be related to an abnormality in membrane function for these cells whereby cation permeability may increase [31]. The changes in all the three phospholipid fraction have been found to be associated with abnormal Na⁺/K⁺ flux which may be related to membrane bound (Na⁺/K⁺)ATPase activity. The decrease of individual phospholipid of erythrocyte membrane in jaundiced neonates may be explained by the fact that lack of cellular glutathione [4] in these patients creates a high flux of oxidants within the cell which oxidises the fatty acids of the phospholipids comprising the
membrane. It is also evident from Table 2 that the membrane bound (Na\(^+\)+K\(^+\))ATPase activity has been altered in jaundiced neonates. Cell membrane (Na\(^+\)+K\(^+\))ATPase has a spatial localization across the membrane and is suggested to have a two step mechanism of action\(^2\). In the first step the phosphorylation of the enzyme on the inner face of the membrane is activated by Na\(^+\); the second step involves the dephosphorylation on the outer surface of the membrane – the process is activated by K\(^+\) ion. Additionally, it has been recognized that the active centre of the (Na\(^+\)+K\(^+\))ATPase is localized on the interior of the membrane (from where Na\(^+\) activates the ATPase)\(^3\). Since phosphatidyl serine (PS) is the only acidic phospholipid present in significant amounts in all mammalian plasma membranes, several authors\(^34\) have concluded that this phospholipid has a specific importance for the lipid protein interaction and the functioning of (Na\(^+\)+K\(^+\))ATPase system. The PS is located in the human R.B.C. in the inner surface of the bilayer and the substrate catalytic side of (Na\(^+\)+K\(^+\))ATPase system on the inside of the membrane. The results from Table 2 indicate significant increase in (Na\(^+\)+K\(^+\))ATPase activity in jaundiced neonates; this increase in activity can be explained in terms of enhanced phosphorylation step and thereby increasing ATPase activity leading to the depletion of PS level (Table 2). It is further observed that total serum cholesterol content in jaundiced neonates increases to a significant extent compared to age matched control (Results not shown in tables). This can be explained in the light of the theory that mature erythrocytes are unable to synthesize lipids de novo, lipids bound to the R.B.C. membrane are in exchange equilibrium with the lipoprotein bound lipids of the serum\(^5\). It is evident from the present study that erythrocyte membrane permeability is significantly altered in jaundiced neonates. This alteration in permeability characteristics creates a definite change in R.B.C profile and its microenvironment.

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References
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