REVIEW OF LITERATURE
While nephrology is but a toddler in the brief history of compartmentalisation of medical disciplines, conceptualisations of the functions and diseases of the kidney have existed since antiquity, developed over time and still continue to unfold.

HISTORICAL EVENTS

An association between oedema and renal disease was observed long ago during the days of the Roman Empire by Ruphous of Epheus, while describing the clinical association of sclerosis of the kidneys, he observed that the afflicted patients had no pain and urinated little [Major, 1954]. By 1722, Zuinger had described progression to ultimate high output failure with sclerosed kidneys. The term "Nephrotic Syndrome" was introduced by Calvin and Goldberg in 1931. This term gained increasing acceptance in the paediatric literature through the 1930's and began appearing in the internal medicine literature in the late 1940's. Schreiner (1971) defined nephrotic syndrome to be 'a clinical entity with multiple causes and characterised by increased glomerular permeability manifested by massive proteinuria and lipiduria'.

NEPHROTIC SYNDROME

The nephrotic syndrome is not a disease; it is a group of signs and symptoms commonly seen in patients with glomerular disease; that are characterised by a marked increase in capillary wall permeability to serum proteins in addition to glomerular inflammatory changes (like MPGN etc). The primary abnormality in nephrotic syndrome is the excretion of large amounts of (greater than 3.5g/day) of protein in urine. The other manifestations that may occur secondary to proteinuria include hypoalbuminaemia, oedema, hyperlipidaemia and lipiduria. Although an interrelationship between some of these findings was recognised as early as the fifteenth century [Arneil, 1971],
The term nephrosis first achieved widespread acceptance in the early part of this century, when Volhard and Fahr employed it as one of the major divisions of bilateral kidney disease [Schnaper and Robson, 1985]. Later developments, notably, with the advent of percutaneous renal biopsy, have facilitated further delineation of the many forms of kidney disease that result in nephrotic syndrome. In contrast to nephritic syndrome, the onset of nephrosis is usually insidious, gross hematuria and red cell casts are infrequent, and renal function is often normal at the time of presentation.

AETIOLOGY / CAUSES

The list of diseases that may cause the nephrotic syndrome is extensive and includes virtually every disorder that may affect the glomerulus. A classification of the causes of nephrotic is depicted in the table 1. This classification of the causes of nephrotic syndrome is not meant to imply that all patients in any one group have the same disease process. In most of these conditions, the pathogenesis remains poorly understood. Pathologic findings may represent a common end result of many different pathological mechanisms. However, all these diseases share a common denominator in that each causes proteinuria of sufficient severity to produce hypoproteinaemia. Typically, when the serum albumin concentration falls below a critical level of approximately 2g/dl, the other clinical features of nephrotic syndrome appear. The primary (Idiopathic) glomerular diseases associated with nephrotic syndrome, as well as, the diseases secondary to infections or drug causes are discussed below.

PRIMARY / IDIOPATHIC NEPHROTIC SYNDROME

This diagnosis is arrived at by exclusion of known cases such as infections, drug exposure malignancy, multi-system disease, or hereditary disorders. The exclusion of these disorders may, at times, prove difficult since they may exist in covert forms (especially cancer and hepatitis B). The idiopathic forms are further classified according to the morphologic features of
### TABLE 1
CLASSIFICATION OF THE CAUSES OF NEPHROTIC SYNDROME

<table>
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<tr>
<th>PRIMARY GLOMERULAR DISEASE</th>
<th>SECONDARY TO OTHER DISEASES</th>
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</thead>
<tbody>
<tr>
<td>A. Minimal change disease*</td>
<td>A. Inflections</td>
</tr>
<tr>
<td>B. Mesangial proliferative glomerulonephritis</td>
<td>1 Post-streptococcal glomerulonephritis*</td>
</tr>
<tr>
<td>C. Focal and segmental glomerulosclerosis*</td>
<td>2 Malaria</td>
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<tr>
<td>D. Membranous glomerulonephritis</td>
<td>3 Leprosy</td>
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<tr>
<td>E. Membranoproliferative glomerulonephritis</td>
<td>4. Hepatitis B</td>
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<tr>
<td>1. Type I</td>
<td>5. HIV infection and AIDS*</td>
</tr>
<tr>
<td>2. Type II</td>
<td>B. Hypersensitivity Reactions</td>
</tr>
<tr>
<td>3. Other variants</td>
<td>1 Drugs</td>
</tr>
<tr>
<td>F. Other uncommon lesions</td>
<td>- Heavy metal compounds like gold and mercury, other drugs</td>
</tr>
<tr>
<td>1 Crescentic glomerulonephritis</td>
<td>- like penicillamine etc</td>
</tr>
<tr>
<td>2 Focal and segmental proliferative glomerulonephritis</td>
<td>- Heroin addiction</td>
</tr>
<tr>
<td></td>
<td>2 Bee stings*, snake bite, poison ivy</td>
</tr>
<tr>
<td></td>
<td>C. Systemic Diseases</td>
</tr>
<tr>
<td></td>
<td>1 Systemic Lupus Erythematosus (SLE)</td>
</tr>
<tr>
<td></td>
<td>2 Diabetes mellitus</td>
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<tr>
<td></td>
<td>3 Amyloidosis</td>
</tr>
<tr>
<td></td>
<td>D. Malignancy</td>
</tr>
<tr>
<td></td>
<td>1 Carcinomas</td>
</tr>
<tr>
<td></td>
<td>2 Myeloma</td>
</tr>
<tr>
<td></td>
<td>3. Hodgkin's disease</td>
</tr>
<tr>
<td></td>
<td>E. Circulatory Disturbances</td>
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<tr>
<td></td>
<td>1. Renal vein thrombosis</td>
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<tr>
<td></td>
<td>2 Constrictive pericarditis</td>
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<tr>
<td></td>
<td>F. Hereditary Diseases</td>
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<tr>
<td></td>
<td>1 Alport's disease</td>
</tr>
<tr>
<td></td>
<td>2 Fabry's disease</td>
</tr>
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<td></td>
<td>* Most common</td>
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* Most common
renal biopsy (Table 2). Performance of a renal biopsy, at least among adults, is required for the accurate classification of idiopathic nephrotic syndrome, for the formulation of a rational plan of treatment, and for the estimation of the likelihood of subsequent progression to renal failure. Children need not always be subjected to renal biopsy (Indications for their biopsy given in Appendix 1) since clinical study can often lead to an accurate diagnosis. The idiopathic forms are further classified according to the morphologic features of renal biopsy and are discussed below.

A. Minimal change nephrotic syndrome (MCNS)

Minimal change disease is a condition in which nephrotic syndrome is accompanied by no apparent change in glomeruli by light microscopy. Its other synonyms, lipoid nephrosis and foot process disease, are descriptive terms for fatty change in the tubules and electron microscopic appearance of diffuse epithelial foot process effacement, respectively. This is the commonest cause of nephrotic syndrome in young children below the age of 6 years with preponderance in boys (ratio of boys to girls 2:1). Peak prevalence is between 6 and 8 years. This lesion is not rare in adults, representing 15 to 20% of idiopathic nephrotic syndrome in patients over 16 years of age. There is slight predilection for males. Typically, the patients present with overt nephrotic syndrome, normal blood pressure, and normal or slightly reduced glomerular filtration rate (GFR). Urinary protein is typically highly selective in children (e.g., it contains principally albumin and minimal amounts of high-molecular-weight plasma proteins like IgG, alpha\textsubscript{2} macro globulin, or complement 3) but is variable in adults. The pattern of protein excretion indicates a major 'charge-selective' defect in permselectivity.

Since the aetiology and pathogenesis are unknown, treatment is empirical and symptomatic. Glucocorticoids enhance the natural tendency for this disease to undergo spontaneous remission. Daily or alternate-day therapy seems to be equally effective; the later is associated with fewer complications. Because of high probability of this disease, many paediatricians treat the...
<table>
<thead>
<tr>
<th>Lesion</th>
<th>Morphology*</th>
<th>Approximate prevalence in children/adults, %</th>
<th>Common clinical/lab features</th>
<th>Response to therapy</th>
<th>Likelihood of maintaining renal function $</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimal change disease</td>
<td>Normal or very mild proliferation</td>
<td>Negative-trace IgM, Foot process fusion, no deposits</td>
<td>70-80/15-20</td>
<td>Highly selective proteinuria, decreased IgG, increased IgM</td>
<td>Steroids ++, cytotoxic drugs-(cyclophosphamide, chlorambucil), Cyclosporine++, Levamisole+, Frequent relapses.</td>
</tr>
<tr>
<td>Mesangial proliferative</td>
<td>Diffuse mesangial proliferation</td>
<td>Negative of variable mesangial IgM, IgG, Mesangial deposits</td>
<td>15-20/5-10</td>
<td>Hematuria</td>
<td>Steroids±</td>
</tr>
<tr>
<td>Lesion</td>
<td>LM</td>
<td>EM</td>
<td>IFM</td>
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<tr>
<td>Focal and segmental glomerulosclerosis (with hyalinosis)</td>
<td>Foot process fusion, sclerosis, hyalinosis, lipid deposits</td>
<td>Mesangial proliferation, lobular change, interposition</td>
<td>Mesangial nodules</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Membranoproliferative Glomerulonephritis Type I</td>
<td>Subendothelial deposits</td>
<td>Variable IgG, IgM</td>
<td>Mesangial proliferation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Membranoproliferative Glomerulonephritis Type II</td>
<td>Intramembranous deposits</td>
<td>Mesangial proliferation, interposition</td>
<td>Mesangial proliferation, interposition</td>
<td></td>
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<table>
<thead>
<tr>
<th>Likelihood of maintaining renal function</th>
<th>Response to therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>10/10-20%</td>
<td>Steroids+, Cytotoxic drugs, Cyclosporine+</td>
</tr>
<tr>
<td>8/&lt;5</td>
<td>Hematuria</td>
</tr>
<tr>
<td>3/&lt;5</td>
<td>Hematuria</td>
</tr>
<tr>
<td>50</td>
<td>Steroids (?), Anticoagulants (?), Cytotoxic drugs (?), Antithrombotic s+</td>
</tr>
<tr>
<td>40</td>
<td>Steroids+, Cytotoxic drugs-</td>
</tr>
</tbody>
</table>

Source: Glassock and Brenner, 19, Chapter 240. Major Glomerulopathies, Hansons Textbook of Medicine.

*LM=light microscopy, IFM=immunofluorescence microscopy, EM=electron microscopy.

Response to therapy: ++=highly responsive, +=variable responsive, ±=occasionally responsive, -=unresponsive.

Present patients maintaining sufficient renal function to obviate need for chronic dialysis of transplant within 5 years.
children without an initial renal biopsy. A complete steroid response in such circumstances is indicative of the underlying minimal change disease in children.

B. Mesangial proliferative glomerulonephritis

This is another important cause of nephrosis in children and young adults. As the name implies, it is characterised by two histological features – increase in the cellularity of the mesangium associated with increased lobulation of the tuft, and irregular thickening of the capillary wall. In addition, the precise nature of the proliferating cells is not clearly understood but may represent combinations of proliferating mesangial cells, endothelial cells and infiltrating mononuclear cells. Deposits of proteinaceous material, if seen, are confined to the mesangial areas. It is observed that the glomerular involvement is usually reasonably uniform. This lesion accounts for approximately 5% of idiopathic nephrotic syndrome in adults and 5-10% in children. It is more common in older children and young adults. Males are affected slightly more than females. The presence of mesangial immunoglobulin deposits and circulating immune complexes in some patients suggests an immune-complex pathogenesis. Patients with this lesion, who have remission of proteinuria following treatment with glucocorticoids tend to do well, with little inclination toward progressive renal insufficiency. On the other hand, some patients, particularly those with steroid unresponsiveness and superimposed focal and segmental glomerulosclerosis on the initial biopsy, have poor prognosis and often develop renal failure within 5 to 10 years after diagnosis.

C. Focal and segmental glomerulosclerosis with hyalinosis (focal sclerosis) (FSGS)

This lesion is characterised by sclerosis (solidification) and hyalinosis of some, but not all, glomeruli (hence the term focal). Hyalinosis refers to collection of eosinophilic, homogenous, hyaline material present on the inner aspect of a sclerotic peripheral capillary loop. Among the affected glomeruli,
only portions of the glomerular tuft are abnormal (hence segmental) and reveal diffuse epithelial foot process effacement. This lesion accounts for about 10 to 15% of cases of idiopathic nephrotic syndrome among children and is more common in adults, particularly between the ages 16 and 30yrs. Males are affected more often than females. The cause and pathogenesis of focal sclerosis are unknown. A variety of mechanisms have been postulated including immunologic, toxic, biochemical and hemodynamic disturbances. A systemic process is suggested by the high prevalence of recurrent disease in renal allografts (30-40%). There is little tendency for spontaneous remission, except among children. Decline in the GFR is seen albeit at variable rates. Hyperlipidaemia, often severe, is characteristic of focal sclerosis and probably deserves aggressive therapy with hypolipidaemic agents (like lovastatin). A subset of patients with focal sclerosis, heavy proteinuria and profound hypoalbuminaemia, progress rapidly to end-stage renal failure.

D. Membranous glomerulonephritis

This lesion is characterised by widespread thickening of the glomerular capillary wall and is a major cause of nephrotic syndrome in adults. During the initial stage of this lesion, all glomeruli may appear to be normal by light microscopy, but as the disease progresses, deposition of immune complexes occur, and new basement membrane like material is produced, that causes the capillary wall to thicken. Eventually, increased amounts of basement membrane material project toward the urinary space, giving the appearance of 'spikes'. There is little proliferation of capillary endothelial or mesangial cells although mesangial sclerosis may occur in an advanced cases. This disorder accounts for 30 to 40% of cases of idiopathic nephrotic syndrome in adults but is rare in children. Men are affected more often than women. Blood pressure and GFR tend to be normal in the early course of this disorder, making it difficult to distinguish membranous glomerulonephritis from minimal change disease on clinical grounds. Spontaneous complete remissions of nephrotic syndrome are common in children and occur in 20-40% of adults. Progressive
renal failure is seen only in 20-30% of patients, usually 3 or more years after diagnosis.

E. Membranoproliferative glomerulonephritis (MPGN)

This disorder is characterised by proliferation of mesangial cells. The mesangial matrix synthesis is increased as well. The glomerular capillary wall is irregularly thickened, by virtue of the mesangial extensions and attendant synthesis of basement membrane like material. This group of disorder is also known as mesangiocapillary glomerulonephritis. Aetiology of this lesion is unknown though in some cases there is evidence of preceding streptococcal infection. Based on ultrastructural, immunofluorescence and pathogenetic mechanisms, three types of MPGN are recognised. Type I or classic form is an example of immune complex disease and comprises more than 70% cases. It is characterised by immune deposits in the sub endothelial position. Type II or dense deposit disease - In this the capillary wall thickening is due to the deposition of electron-dense material in the lamina densa of the GBM. Type III is rare and shows complex deposits in the GBM and on the epithelial as well as endothelial side of GBM. Clinically there are many similarities between the main forms of MPGN. The most common age at diagnosis is between 15 and 20 years. Approximately 50% of the patients present with nephrotic syndrome; about 30% have symptomatic proteinuria; and 20% have nephrotic syndrome at presentation. With time, majority of the patient's progress to renal failure, while some continue to have proteinuria, hematuria and hypertension with stable renal function. Spontaneous remissions are uncommon and long-term, alternate-day prednisone therapy may delay the progression of the disease.

OTHER FORMS OF IDIOPATHIC NEPHROTIC SYNDROME

In a small percentage of adults and children with idiopathic nephrotic syndrome other lesions are encountered on renal biopsy. These include crescentic glomerulonephritis and focal and segmental proliferative
glomerulonephritis. The clinical characteristics, natural history and response to treatment of these lesions are not well defined.

NEPHROTIC SYNDROME CAUSED BY INFECTIOUS AGENTS, DRUGS OR CHEMICALS

Table 2 lists down the common infectious and drug-related causes of nephrotic syndrome. In many instances, nephrotic syndrome abates following cure of infection or withdrawal of the offending medication. In patients receiving gold therapy for rheumatoid arthritis or in those exposed to inorganic, organic or to penicillamine, membranous glomerulonephritis is usually the lesion responsible for nephrotic syndrome. Human immunodeficiency viral infection with or without full-blown AIDS may be associated with nephrotic syndrome and progressive renal failure. Nephrotic syndrome is known to follow immunization and antiserum treatment of tetanus or snakebite.

Nephrotic syndrome is caused through various causes as discussed in table 1. Various mechanisms through which glomerular inflammation occurs and the consequences that follow later will be dealt below.

MECHANISMS OF GLOMERULAR INFLAMMATION

Both humoral and cell-mediated mechanisms play a part in the pathogenesis of glomerular inflammation (Figure 1) [Cibrik and Sedor, 1997]. Two basic mechanisms of antibody-mediated glomerular injury have been identified.

First: The antibodies can bind either to a structural component of the glomerulus or to material that is not intrinsic to the glomerulus but is there because of its physicochemical characteristics. For example: In patients with SLE, histone-DNA complexes, which can bind to glomerular cell surfaces and basement membrane, is an example of 'planted' antigens that could be target of anti-DNA antibodies [Jacob et al., 1989]
FIGURE 1
MECHANISMS OF GLOMERULAR INFLAMMATION

Antibody deposition

Cell-mediated immune mechanism

Complement activation

Influx of circulating leukocytes

Hemodynamic alterations

Genetic factors

Cytokine/growth factor synthesis

On-off switch

Glomerulonephritis activation of resident cells change in matrix

Persistent inflammation

Exit of anti-inflammatory molecules and leukocytes

Scarring

Resolution

Source: Cibrik and Sedor [1997]
Second: The circulating antigen-antibody complexes form, escape the clearance by the reticuloendothelial system, and are deposited in the glomerulus. A number of exogenous and endogenous antigens have been identified in circulating immune complexes and implicated in the pathogenesis of human glomerulonephritis [Wilson, 1991].

Several studies have suggested that the activation of cell-mediated immunity can also induce glomerular injury. First, the adoptive transfer of sensitised T lymphocytes to rat treated with subnephritogenic doses of antibody results in glomerular hypercellularity due to proliferation of resident glomerular cells and an influx of mononuclear leukocytes [Bhan et al., 1979]. Second, in chickens unable to mount an antibody response because of bursectomy, severe proliferative nephritis develops after immunization with GBM [Bolton et al., 1984]. Finally, granulomatous nephritis can be reproduced in previously unexposed recipients by adoptive transfer of T cells but not by passive administration of antibody [Rennke et al., 1994]. In humans, T cells have been identified in both proliferative and non-proliferative glomerulopathies (Main and Atkins, 1995). Treatment with cyclosporine, an inhibitor of T-cell function, is effective for some glomerular diseases, suggesting that the experimental findings also apply to glomerular injury in humans [Catran et al., 1995].

After the initiation of glomerular injury, a number of proinflammatory mediator pathways are activated in both infiltrating cells and resident glomerular cells [Cibrik and Sedor, 1997]. Complement activation, influx of circulating leukocytes, cytokine synthesis, release of proteolytic enzymes, activation of the coagulation cascade and generation of proinflammatory lipid mediators have been demonstrated in experimental glomerulonephritis and to a more limited degree, in human disease [Johnson, 1994; Couser, 1993]. Resident cells within the kidney become activated after injury and participate in subsequent destructive and restorative processes [Johnson, 1997; Johnson, 1994; Sedor et al., 1993]. In addition, the amount and composition
of extracellular matrix are modified [Johnson, 1994]. Matrix remodelling in response to injury generates signals that are different from those transmitted by normal glomerular matrix and may facilitate the activation and proliferation of both resident and infiltrating glomerular cells.

Many other processes control the amplification, progression, or resolution of glomerulonephritis. The adaptive hemodynamic alterations in the remaining functional glomeruli cause hyperfiltration, intraglomerular hypertension and abnormal intravascular stress and shear. These altered physical forces can exacerbate the ongoing glomerular injury [Johnson, 1994; Bernner et al., 1996]. Depending on the cells affected or programmed cell death; all these may have a crucial role either in the resolution of glomerulonephritis or in glomerular scarring (Savili, 1996).

So when the glomerulus is attacked by any kind of infection, it leads to the generation of inflammatory mechanisms, which in turn alters the glomerular architecture. Once the glomerular architecture is altered, it leads to increased losses of proteins in urine leading to proteinuria. And this is what happens during 'nephrotic syndrome'. The various causes leading to the development of nephrosis have been dealt with earlier. Hence, any of these causes can precipitate the development of nephrotic syndrome.

**DEFINITION**

Nephrotic syndrome is defined as a clinical condition in which >3.5g/1.73m$^2$/day of proteins are excreted in urine. The clinical manifestations are proteinuria, hypoalbuminaemia, hyperlipidaemia and oedema. These abnormalities are the consequences of excessive glomerular leakage of plasma proteins into the urine. The defects in the charge- or size-selective barriers of the glomerular capillary wall that underline the excessive filtration of plasma proteins can arise as a consequence of a variety of disease processes (as discussed earlier), including immunological disorders, toxic injuries, metabolic abnormalities, biochemical defects and vascular disorders.
Thus, nephrotic syndrome is a common end point of a variety of disease processes that alter the permeability properties of the GBM or glomerular capillary wall. Proteinuria is the hallmark of the nephrotic state.

PROTEINURIA

Virtually every abnormality observed in primary nephrotic syndrome could be traced directly or indirectly to the occurrence of proteinuria. This is not only a marker of glomerular damage but is toxic to the kidneys, as shown by elegant studies of Eddy et al., 1995 and emphasised by the National Kidney Foundation Initiative, PARADE (Proteinuria, Albuminuria, Risk, Assessment, Detection, Elimination) [Keane and Eknoyan, 1999]. It has been known for decades that the prognosis and course of glomerular diseases were closely correlated with the level of proteinuria. Patients with less than a gram of proteinuria had a slowly progressing disease, whereas those with increasing quantities of protein excretion had an increasingly accelerated course to end-stage renal disease. Increased proteinuria reflects damage to the glomeruli and increases the development of FSGS and directly causes injury to the tubules [Eddy et al., 1995].

PATHOPHYSIOLOGY OF PROTEINURIA

For understanding the pathophysiology of proteinuria, it is important to review first the handling of the proteins by normal kidney.

Glomeruli are normally perfused with plasma containing over 60,000g of proteins per day, but less than 150mg of protein is excreted in the final urine. The filtration barrier, which includes the endothelial cells, glomerular basement membrane (GBM), epithelial cells and slit diaphragms, all these restrict the transcapillary passage of proteins on the basis of their size, shape and electrical charge. The size barrier is primarily at the level of the endothelial cells and GBM. It restricts the filtration of molecules between about 18 and 42 Å and effectively prevents filtration of neutral molecules larger than 42 Å. Circulating proteins such as albumin (36 Å) are further...
restricted from crossing the glomeruli by an electrical charge barrier. The glomerular filter contains negatively charged sialoglycoproteins [Mohos and Skoza, 1967] present at regularly spaced intervals in the lamina rarae of the basement membrane [Kanwar and Farquhar, 1979] at the endothelial fenestrae [Latta and Johnson, 1976] and lining the epithelial podocytes [Latta et al., 1975]. Collectively, these comprise the glomerular polyanion (Figure 2). The presence of such negative charge sites are believed to be responsible for both the facilitated transport of polycations [Bohrer, 1978] and the restricted transport of polyanions [Chang et al., 1975] relative to that of neutral molecules of comparable size. Thus the determinants of glomerular permeability for a given particle are steric hindrance, glomerular hemodynamics and electrostatic charge [Brenner et al., 1977]

PATHOPHYSIOLOGY OF ABNORMAL URINARY PROTEIN EXCRETION

Increases in the urinary excretion of proteins result from increases in their filtered load, due to alterations in the permselectivity of the glomerular capillary wall or defects in their tubular uptake. The majority of the experimental studies using inert test probes (like Dextran, Ficoll) have shown that the alteration of the selectivity of the glomerular capillary wall was a combination of the loss of charge restriction and size restriction. The reduction in the restrictive properties of the glomerular barrier leads to proportionally greater increases in the filtered loads of albumin and high molecular weight proteins than that of low molecular weight proteins; the glomerular permeability of the latter is already very high and cannot increase further, whereas even a small increase in the glomerular permeability to macromolecules leads to very significant increases in the filtered load of larger proteins.

In addition, the development of nephrotic syndrome is also characterised by numerous morphological changes in the podocytes. The most important characteristic structural alteration is retraction and effacement.
of podocyte foot processes, resulting in the formation of a diffuse cytoplasmic sheet along the GBM (Figure 3). Other structural changes include cell swelling, occurrence of occluding junctions with apical displacement of the slit diaphragms (located in the filtration slits between foot processes of adjacent podocytes) and frequently detachment of the podocyte from the underlying GBM [Ito et al., 1986; Caulfield et al., 1976; Rayn and Karnovsky, 1975]. Detachment of effaced foot processes from the GBM is generally considered the most severe structural manifestation of nephrotic syndrome (Figure 3). Several studies have now clearly demonstrated that the detachment of the podocyte from the GBM results in leakage of proteins across the GBM at the site of detachment [Laurens et al., 1995; Whiteside et al., 1989; Messina et al., 1987; Kanwar and Rosenzweig, 1982]. Based on these findings, the podocytes appear to form a significant portion of the kidney's filtration barrier, and the proteinuria seen during nephrosis is now thought to result directly from the leakage of massive amounts of protein across the GBM at these sites of podocyte detachment.

In addition to the dramatic changes in the foot process structure during nephrotic syndrome, significant alterations also occur in the filtration slits and slit diaphragms. Foot process effacement results in a decrease in the filtration slit frequency along the GBM and has been associated with narrowing of the filtration slits and development of tight junctions in between the foot process [Kurihara et al., 1992]. These structural alterations in the filtration barrier may act together to reduce the overall glomerular filtration, a finding reported in human nephrotic syndrome [Guasch and Myers, 1994]. Support for the importance of podocytes in the maintenance of the glomerular filtration barrier include the mathematical calculations which suggest that the filtration slits provide about 50% of the hydraulic resistance of the glomerular capillary wall, and studies have shown the induction of proteinuria following treatment of rats with an antibody directed primarily against a slit membrane-associated antigen [Blantz et al., 1994]. Within the filtration slits the majority of hydraulic
FIGURE 3

COMPARISON OF PODOCYTE FOOT PROCESS STRUCTURE IN THE NORMAL STATE AND DURING NEPHROTIC SYNDROME

Fig 3A: Podocyte foot process in normal state

Fig 3B: Podocyte foot process during nephrotic syndrome

Note: Arrow - Podocyte foot process, Arrowhead - GBM
Source: Smoyer and Mundel [1998]
resistance is thought to be provided by the slit diaphragms themselves [Drummond and Deen, 1994].

Induction of foot process effacement by perfusing the kidney with the polycation protamine sulfate has been reported to cause apical displacement of the slit diaphragms and development of true tight junctions between foot processes [Kurihara et al., 1992]. Importantly, the ZO-1 protein co-localises acutely with both the newly formed tight junctions and the displaced slit diaphragms [Kurihara et al., 1992] and is phosphorylated on tyrosine residues. These findings suggest that the phosphorylation of tight junction-associated proteins may be part of a signalling pathway responsible for slit diaphragm displacement and the formation of tight junctions in the podocytes during foot process effacement.

The negative charge of the podocyte (glycocalyx) also appears to have a critical role in the regulation of podocyte foot process structure. Several studies have reported that infusion of polycations (protamine sulfate) into rats resulted in both neutralization of the negative surface charge and effacement of podocyte foot processes [Bridges et al., 1991; Hunsicker et al., 1981; Kerjaschki, 1978; Seiler et al., 1977; Seiler et al., 1975]. Induction of nephrotic syndrome in the rats with puromycin aminonucleoside (PAN) has also been reported in some studies to be associated with reduced negative charge of the glomerular filtration barrier. Together these reports suggest that the maintenance of the negative charges on the GBM and podocytes is important for the maintenance of normal glomerular filtration and foot process structure, and that the loss of charge selectivity may lead to selective increase in albumin excretion (and that the reduction in these negative charges may have an important role in the development of nephrotic syndrome).

ROLE OF GLOMERULAR ELECTROSTATIC CHARGE IN NEPHROSIS

There is a significant body of evidence suggesting that proteinuria in MCNS and its variants result from the alterations in the glomerular charge.
sites. This proteinuria is highly selective and consists primarily of albumin, which carries a negative charge under normal conditions. However, the renal clearances of the proteins carrying other charges are not increased comparably despite massive proteinuria. Renal biopsy material from patients with nephrotic syndrome provides an explanation for this observation, since there is decreased staining for glomerular polyanion [Blau and Haas, 1973; Carrie et al., 1981; Mahan et al., 1985]. Indeed, studies in MCNS patients have suggested that albuminuria occurring due to reduction of fixed negative charge is approximately 50% [Bridges et al., 1982]. Depletion of polyanion also has been documented in congenital nephrotic syndrome [Vernier et al., 1983], where a semi quantitative technique has revealed a significant decrease in the glycosaminoglycan content of GBM. These reports indicate that disparate mechanisms can cause depletion or alteration of charge sites in nephrosis.

The cause of the depletion or neutralization of negative charge in human disease is unknown. Intraglomerular release of platelet factor 4, which has been shown to bind to glomerular polyanion by an ionic reaction [Barnes et al., 1984], could cause proteinuria by a mechanism analogous to that seen with polycation infusions. Studies in the patients suggest that the neutralization of the anionic charges may be systemic in nature [Levin et al., 1989; Levin et al., 1985] rather than confined to the kidneys (though these changes in the anionic charges may be systemic in nature but its effects can be noticed in the kidneys also). This could result from effects of a protease present in the circulation [Bakker and van Luijk, 1989]. A variety of immunologic factors represent additional potential mechanisms for inducing albuminuria in MCNS. Once initiated, proteinuria could be perpetuated by additional mechanisms, such as alterations in the arachidonic acid metabolism, which increases the thromboxane production in the kidneys [Remuzzi et al, 1985].
Defects in the glomerular architecture leads to proteinuria in nephrotic syndrome and how it happens was discussed earlier, now let's see how the kidneys handle these abnormal levels of proteins. Hence, let's observe the renal tubular handling these abnormal levels of filtered proteins.

**RENAL TUBULAR HANDLING OF AN ABNORMAL LOAD OF FILTERED PROTEINS**

In normal physiological conditions, the high molecular weight (HMW) proteins are restricted almost completely from reaching the tubular lumen because of the impermeability of the glomerular barrier (Figure 4a, b, c, d). But during diseased states, i.e. when impairment of the charge and selectivity of the glomerular capillary wall increases the filtration of the proteins of intermediate and HMW, these proteins compete among themselves, and with the low molecular weight (LMW) proteins, in the process of reabsorption by the epithelial cells of the proximal tubule. If this mechanism is saturated, some of the filtered proteins of all sizes, including those of LMW, appear in the urine (Figure 4a, b, c, d). Unfortunately, the fraction of the different proteins that escapes reabsorption is not proportional to the filtered fraction, since it is becoming evident that the reabsorption process is not specific and unselective, and that even the charge of the various proteins influences such processes, like IgG being more easily reabsorbed than albumin because of its positive charge.

When the load of the filtered proteins escaping the glomerular barrier is massive, the epithelial cells of the proximal tubule, subjected to a continuous overload, may progressively lose their integrity and cause morphological changes. As a result, the reabsorption of all proteins, including the physiological reabsorption of LMW proteins, is increasingly impaired, leading to the urinary excretion of a progressively larger fraction of LMW proteins (Figure 4c and 4d). It has been found that such impairment of the reabsorption of LMW proteins, the glomerular disease with severe proteinuria, correlates with the extent of the injury of the tubular cells.
FIGURE 4

SCHEMATIC REPRESENTATION OF TRANSGLOMERULAR TRANSFER OF PLASMA PROTEINS

Source: D'Amico and Bazzi (2003)
FIGURE 4
SCHEMATIC REPRESENTATION OF TRANSGLOMERULAR TRANSFER OF PLASMA PROTEINS

Source: D'Amico and Bazzi [2003]
and with the overall tubulointerstitial damage [Remuzzi et al., 1997; D'Amico et al., 1995; Magi, 1995; Burton and Walls, 1994; Williams and Coles, 1994; Widstam-Attorps et al., 1992] And later on, if this continues, it may lead to progressive renal failure.

Before severe and progressive proteinuria leads these patients to renal failure, attention has be to be focused on more important things like the consequence of proteinuria, which is hypoproteinaemia.

**HYPOPROTEINAEMIA**

It is generally accepted that the central feature of nephrotic syndrome, irrespective of its underlying cause, is hypoproteinaemia resulting from the urinary losses of proteins, mainly albumin (hypoalbuminaemia). Among all the proteins, albumin is the only protein that has been extensively investigated in nephrotic syndrome.

The hepatic albumin synthesis is normally 12 to 14 g/day in adults and may increase in nephrotic syndrome but this synthesis can be limited by various factors, including age, poor nutritional status, and liver disease [Coggins, 1982]. During nephrotic syndrome, the serum concentration of albumin decreases to less than 3g/dl when the rate of urinary protein loss and renal catabolism of filtered albumin exceeds the rate of hepatic synthesis. Thus, it is seen that some patients may exhibit significant hypoalbuminaemia with less amount of proteinuria, while in some other patients even though they excrete large amounts of proteins, are better able to maintain their serum albumin levels. The amount of albumin lost daily in the urine of the nephrotic patients is usually lower than the normal daily hepatic synthesis of albumin, and thus proteinuria (albuminuria) is a necessary contributor to the development of hypoalbuminaemia. But in some patients, the urinary protein losses may exceed the normal rate of synthesis of albumin by liver. It is therefore, not clear why hypoalbuminaemia should occur in those patients.
with lesser degrees of proteinuria. However, there are three possible explanations:

1. Synthesis of albumin could be diminished/increased
2. Catabolism of albumin could be enhanced, or
3. Non-renal losses of albumin could occur.

Let us see how albumin is metabolised during nephrosis.

**ALBUMIN METABOLISM IN NEPHROSIS**

Since nephrotic syndrome is defined by the urinary loss of 3.5g of proteins per day with resulting hypoalbuminaemia [Orth, 1997], it is useful to review both the homeostatic response to and the physiological consequences of the loss of this important serum protein in detail.

In health, the basal rates of both albumin synthesis and catabolism are 12 to 14 g/day (Rothschild *et al.*, 1977). Slightly less than half of the total body albumin stores are in the vascular pool with the rest being in the interstitial fluid, mostly in skin and muscle. When external albumin loss occurs, these extravascular pools are rapidly mobilised [Sellers *et al.*, 1966] so that in nephrotic patients most of the albumin are intra-vascular. Mobilisation of extravascular albumin, while effective in reducing oedema formation, is of limited value only, in stabilising the serum albumin concentration after increased external loss. A new steady state can be reached only by an increased rate of albumin synthesis, a decreased rate of albumin catabolism, or both.

(a) Albumin Synthesis in Nephrosis

Although the rate of albumin synthesis may be nearly doubled in some extremely albuminuric patients, this is uncommon. Albumin synthesis may be increased, decreased or normal in the patients with hepatic cirrhosis [Rothschild *et al.*, 1969], is reduced with the hypoalbuminaemia of malnutrition, and is increased only slightly in most patients with nephrotic syndrome [Jensen *et al.*, 1967]. Albumin synthesis by isolated perfused liver
is, however, inversely proportional to the osmotic pressure of the perfusate. It is increased at low osmotic pressure; and is decreased when the osmotic pressure is increased [Kaysen et al., 1986; Oratz, 1976]. Mathews in 1961 determined that the rate of albumin synthesis depended upon the extravascular protein mass or concentration rather than upon the intravascular protein concentration in the rabbits. He theorised that the intravascular pool would be maintained at the expense of the extravascular pool, and that the reduction of the extravascular pool would stimulate albumin synthesis. Thus, the hemodynamically important transfer of albumin from interstitial pools to the vascular compartment for the purpose of maximising the colloid osmotic gradient might also play a key role in triggering an increase in the albumin synthetic rate. Rothschild et al. [1966] and Oratz [1976] theorised that a key interstitial pool of albumin existed in the extravascular compartment of the liver and argued that the processes that reduced the albumin content of that pool would increase the rate of albumin synthesis. Conditions which reduced the content of extravascular albumin in the liver in vivo were able to increase the rate of albumin synthesis. Although the total extravascular albumin pool was depleted in nephrotic animals, the interstitial pool in the liver was not [Oratz, 1976].

There is ample evidence that the liver is stimulated to produce more albumin in nephrosis. Isolated microsomal preparations obtained from the livers of nephrotic rats showed marked increase in the rate of albumin synthesis in vitro. Therefore, although the nephrotics have the capacity to increase the albumin synthesis [Kaysen et al., 1990; Marsh et al., 1966], this capacity seems insufficient to avoid hypoalbuminaemia [Jensen et al., 1967]. Patients undergoing continuous ambulatory peritoneal dialysis, however, do increase the rate of albumin synthesis sufficiently to nearly normalise serum albumin concentration despite similar albumin losses it must be concluded that the nephrotic patients may have specific inability to adequately respond to albumin losses by increasing the rate of albumin synthesis appropriately.
Albumin synthesis is increased both in nephrotic patients and rats [Kaysen et al., 1986] when they are fed high protein diet. The renal excretion of albumin, however, is also increased in both patients and animals during the consumption of a high protein diet. The net results of the two opposing effects of increased protein intake are decrease in the serum albumin concentration. These opposite effects of protein intake may make it difficult for proteinuric animals to rebuild depleted amino acid stores by increasing dietary protein intake. The nephrotic syndrome may result in a metabolic 'double bind'. When the dietary protein intake is restricted, albumin synthesis is not increased despite hypoalbuminaemia. When protein intake is increased, the rate of albumin synthesis increases, but so does the renal clearance of albumin. Any increase in the albumin production will not result in the restoration of body pools of this protein as a consequence of the increased urinary loss.

Hepatic response to other proteins like immunoglobulins

The main metabolic response is mediated by the liver may be exemplified by analysing the plasma immunoglobulin (Ig) concentration. Joven et al., 1997 observed that the plasma immunoglobulins represent 21.8% of the total plasma protein in controls and similar amount of plasma immunoglobulin was found in the nephrotics (22.5%). Plasma levels of IgG significantly decreases as a consequence of urinary losses because of the absence of any counter regulatory response in the form of increased IgG synthesis [Kaysen et al., 1990; Deen et al., 1985; Giangiacomo et al., 1975], and this may account for higher predisposition to bacterial infection [Yokoyama et al., 1985]. The plasma concentration of IgM was found to be normal while the IgA concentration was observed to be higher in nephrotics than in controls, which probably reflects the immunologic basis of the renal disease [Beale et al., 1983].

(b) Albumin catabolism in nephrosis

The absolute rate of albumin catabolism is reduced in hypoalbuminaemic states. Like in most of these conditions, such as
Kwashiorkor, protein losing enteropathy and severe liver disease, the fractional rate of albumin catabolism is also reduced. The fractional rate of albumin catabolism is that fraction of vascular pool catabolised per unit time, and a reduction in this rate is the result of a decrease in the first order rate constant that governs the endogenous breakdown of albumin. Although the absolute rate of albumin catabolism is reduced in nephrosis, the fractional catabolic rate may actually be increased [Katz et al., 1963]. Katz, Bonorris and Sellers [Katz et al., 1963] reasoned that the increased fractional rate of catabolism of albumin in nephrosis was the result of renal reabsorption and catabolism of the protein. They showed that the rate of albumin catabolism was directly proportional to urinary albumin loss and concluded that renal catabolism of albumin contributed significantly to total albumin losses in nephrosis. Katz, Bonorris and Sellers [Katz et al., 1963] used puromycin aminonucleoside (PAN) to produce nephrosis. This agent causes a reduction in single nephron glomerular filtration rate in experimental animals. In addition, the animals eat poorly and lose weight. In nephrotic patients it has been observed that the absolute rate of albumin catabolism is reduced [Reed, 1981]. The decrease in the rate of albumin catabolism is actually greater than the increase in the rate of albumin synthesis in these patients. Although, it is possible that the renal component of albumin catabolism in increased in nephrosis [Park et al., 1984], the absolute contribution of the kidney to albumin catabolism. Regardless of the contribution of the kidney to albumin catabolism in nephrosis, it is clear that the absolute value of albumin catabolism is significantly reduced in both nephrotic patients [Kaysen et al., 1986; Christensen et al., 1981] and animals [Kaysen et al., 1984;]. Increased albumin catabolism is, therefore, unlikely to contribute to the generation or maintenance of hypoalbuminaemia in nephrosis is proposed. It is instructive; therefore, to examine in detail the other potential homeostatic response to urinary albumin loss like the changes in the rate of albumin synthesis.
(c). Non-renal losses of albumin

Non-renal losses of albumin probably occur through the gastrointestinal tract i.e. it occurs through the transudation of albumin across the bowel wall. Even though the evidence is conflicting, in the late 1960's, Jensen et al. (1967) and Yssing et al. (1969) in their studies found no evidence of the increased gastrointestinal losses of protein. But in a later study by Schultze et al. (1980) did show moderate to severe gastrointestinal protein losses in patients with nephrotic syndrome (by using $^{51}$Cr-albumin).

OEDEMA

One of the major consequences of hypoproteinaemia (or hypoalbuminaemia) is oedema formation in these patients. It is defined as an excess accumulation of fluid within the interstitial space, resulting from either retention of excess salt and water or from increased transfer of fluid across the capillary membranes. Therefore, it signifies an increase in the volume of the interstitial fluid in the tissues.

Let us first observe the maintenance of normal pressure gradient during normal conditions:

In normal conditions, the composition of body water changes in humans, as they grow older. The total body water in a newborn child is approximately 78% of body weight and is distributed in the extracellular fluid compartment (45%) and in the intracellular fluid compartment (33%). By the end of the first year, the extracellular fluid compartment contracts to 30% of the body weight and the intracellular compartment expands to 40%. Throughout childhood, the extracellular fluid compartment continues to decrease until the total body water reaches 60-65% of the body weight with the extracellular fluid compartment comprising 20-25% of the body weight [Rai and Fernadese, 1999].

The interstitial fluid formation and absorption in relation to pressure gradients acting across the capillary endothelium, the surface area available
for fluid transfer and the permeability of the capillary membrane to proteins was first described by Starling [Rai and Fernadese, 1999; Starling, 1986].

Normal pressure gradients: The movement of fluid across the capillary bed is regulated by the hydrostatic and the colloid osmotic pressure on either side of the membrane. At the arterial end of the capillary bed, the hydrostatic pressure in the vessels is 25mmHg. The hydrostatic pressure in the interstitial compartment is -7mmHg. The effective pressure promoting movement of the fluid out of the capillaries is therefore 32mmHg. The colloid osmotic pressure of blood is 28mmHg and that of interstitial fluid is 4.5mmHg. The effective colloid osmotic pressure preventing the outward movement of the fluid is 23.5mmHg. The net result is that the fluid moves out of the capillaries into the interstitial compartments. At the venous end of the capillaries, the hydrostatic pressure is 9mmHg. The interstitial fluid pressure is -7mmHg. This countered by the effective colloid osmotic pressure of blood i.e., 23.5mmHg. Therefore, the fluid is reabsorbed at the venous end of the capillaries. A part of the fluid in the interstitial compartment is also absorbed in the form of lymph. Normally, there is a balance between the input and output of the fluid from the interstitial compartment. In some clinical disorders, large quantities of fluid move into the interstitial compartment and less is reabsorbed. This results in two types of oedema, which may be generalised or localised and formation of oedema depends on many reasons, which will be dealt latter.

TYPES OF OEDEMA

1. Generalised oedema

This occurs when the potential for fluid to leave the vascular space occurs in all the vascular beds within the body soft tissue and swelling of most or all the regions of the body are seen. Generalised oedema is usually the manifestation of a primary disorder like renal disease. One of the common causes being nephrotic syndrome.
2. Localised oedema

This occurs when oedema is limited to a particular organ or vascular bed (and it can be easily be distinguished from generalised oedema). Fluids collect locally because of either an increase in the capillary hydrostatic pressure induced by arteriolar dilation, venous obstruction, obstruction to regional lymphatic flow or due to increase in the capillary wall permeability (commonly resulting from either trauma or histamine release). Generalised disturbances such as hypoalbuminaemia may present as localised ankle oedema. One of the most common causes of localised oedema is inflammation. Localised oedema can be found in face and/or eyelids of nephrotic patients.

3. Recurrent oedema

Another third type of oedema i.e. recurrent oedema is observed in many clinical conditions like oedema occurring due to cardiac reasons or it may be drug induced oedema or in renal disorders. In renal disorders, nephrotic syndrome is one the clinical condition in which recurrent oedema occurs.

The formation of oedema depends on many reasons that operate singly or in combination to produce oedema.

MECHANISMS OF OEDEMA FORMATION

Oedema is caused by the mechanisms that interfere with the normal fluid balance of plasma, interstitial fluid and lymph flow. And albumin, because of its relatively small molecular size, is the plasma protein primarily responsible for the generation of the oncotic pressure. Because of the mathematical relationship between the plasma albumin concentration and the oncotic pressure, a decrease in the former, as in nephrotic syndrome, results in an even greater decrease in the oncotic pressure, so that the net driving force for the loss of fluid at the arteriolar end of the capillary bed is increased and return of the fluid at the venous end is reduced. In consequence, fluid accumulates in the interstitial space, initiating oedema formation.
accumulation first occurs where the tissue pressure is the lowest, for example, in the eyelids or the scrotum (and it also appears to be in the most dependent parts of the body, because the venous pressure is highest at these sites and is transmitted to the venous end of the capillaries).

The translocation of the fluid from the vascular to the interstitial fluid spaces as oedema forms should decrease the blood volume. It has been proposed that the physiologic responses precipitated by such a reduction are important factors in producing the massive amounts of oedema often seen in the nephrotic syndrome. These change include the release of the anti-diuretic hormone (ADH), the release of renin with increased production of angiotensin II, and decreases in renal blood flow and GFR. All these change favour the renal retention and positive balances of both sodium and water unless intakes are decreased. Indeed, these patients may exhibit increased thirst, which is probably stimulated by the angiotensin II [and by decrease in the blood volume.

The pathophysiology of oedema formation in nephrotic syndrome is probably more complex than this traditional concept. The following mechanisms may be operating singly or in combination to produce oedema.

1. Decreased plasma oncotic pressure
2. Increased capillary hydrostatic pressure
3. Lymphatic obstruction
4. Tissue factors
5. Increased capillary permeability
6. Sodium and water retention

Pathogenesis and pathophysiology of oedema in nephrotic syndrome is incompletely understood [Feraille et al., 1995; Humphreys, 1994; Perico and Remuzzi, 1993]. But it has been observed that two pathophysiological processes appear to be involved. They are (1) decreased oncotic pressure or disruption of Starling equilibria at the peripheral capillary level and (2) primary
renal sodium retention and water retention [Anderson et al., 1995; Feraille et al., 1995; Humphreys, 1994; Perico and Remuzzi, 1993].

1. Decreased plasma oncotic pressure

The plasma oncotic pressure exerted by the total amount of plasma proteins tends to draw the fluid into the vessel normally. A fall in the total plasma protein level, results in the lowering of the plasma oncotic pressure so that it can no longer counteract the effect of hydrostatic pressure of blood. This results in increased outward movement of fluid from the capillary wall and decreased inward movement of fluid from the interstitial space causing oedema. Hypoproteinaemia usually produces generalised oedema. Out of the various plasma proteins, albumin has four times higher plasma oncotic pressure than globulin so then it is hypoalbuminaemia which results in oedema more often. It has been noticed that a decrease in the plasma oncotic pressure due to hypoalbuminaemia will result in a major shift of intravascular fluid into the interstitial compartment, but this does not occur until the plasma oncotic pressure falls below 8mmHg (normal about 25mmHg). This is because of an accompanying fall in the interstitial oncotic pressure, thus maintaining the transcapillary oncotic pressure gradient. Nevertheless, a very abrupt decline in plasma oncotic pressure, such as with rapidly developing nephrotic syndrome, may not be associated with a corresponding decline in the interstitial oncotic pressure and thereby a major displacement of fluid into the interstitial compartment may occur [Joles et al., 1993; Dorhout-Mees and Koomans, 1990; Geers et al., 1984]. However, in more slowly-developing circumstances, peripheral oedema with the expansion of the extracellular fluid volume will occur even when the plasma oncotic pressure is only modestly reduced, thus, factors other than transcapillary oncotic pressure gradient must be involved in oedema formation.

Numerous studies have demonstrated that oedematous patients with hypoproteinaemia (hypoalbuminaemia) during nephrosis may have normal, decreased or even expanded intravascular volume [Joles et al., 1993;
Dorhout-Mees and Koomans, 1990; Geers et al., 1984. In fact these patients with reduced intravascular volume and nephrotic syndrome usually have relatively rapid development of oedema and minimal glomerular abnormalities. On the other hand, expansion of the extracellular volume combined with normal or increased intravascular volume, points to an important contribution of primary renal sodium and fluid retention in the pathogenesis of oedema in the nephrotic patients.

2. Primary renal sodium and water retention

The normal regulatory mechanism of sodium and water balance will be discussed before describing the mechanism of oedema by sodium and water retention.

Normally, about 80% of the sodium is reabsorbed by the proximal convoluted tubule under the influence of intrinsic renal or extra-renal mechanism.

(a). Intrinsic renal mechanism

This mechanism is activated in response to sudden reduction in the blood volume (hypovolaemia). Hypovolaemia stimulates the arterial baroreceptors, which, in turn sends the sympathetic outflow via the vasomotor centre in the brain. As a result of this, renal ischaemia occurs that causes reduction in the glomerular filtration rate, decreased excretion of sodium in urine and consequent retention of sodium (Figure 5).

(b). Extra-renal mechanism

This mechanism involves the secretion of aldosterone, a sodium retaining hormone, by the rennin-angiotensin-aldosterone (RAA) axis. Renin is an enzyme secreted by the granular cells in the juxta glomerular apparatus. Its release is stimulated in response to low concentration of sodium in the tubules. Its main action is the stimulation of angiotensin, which is \( \alpha_2 \)-globulin, or renin substrate that is present in the plasma. On stimulation, angiotensin I, a decapeptide, is formed in the plasma which is subsequently converted into
angiotensin II, an octapeptide, in the lungs and kidneys. Angiotensin II stimulates the adrenal cortex to secrete aldosterone hormone. Aldosterone increases the sodium reabsorption in the renal tubules and sometimes causes a rise in the blood pressure (Figure 5).

Retention of sodium leads to the retention of water secondarily under the influence of anti-diuretic hormone (ADH) or vasopressin. This hormone is secreted by the cells in the hypothalamus and is stored in the posterior pituitary. The release of this hormone is stimulated by increased concentration of sodium in plasma and hypovolemia. Large amounts of ADH produce highly concentrated urine.

Excessive retention of sodium and water and their decreased renal excretion occur in response to hypovolemia and lowered concentration of sodium in the renal tubules via stimulation of intrinsic renal and extra-renal mechanisms as well as via release of ADH (Figure 5). The classical mechanisms of oedema formation in nephrosis is shown in figure 6. According to this model of oedema formation, when the protein concentration is low, the transcapillary flux of fluid outstrips the ability of the lymphatics to return the ultra-filtrate to the plasma compartment [Guyton and Hall, 1996]. The resultant reduced plasma volume activates the renin angiotensin aldosterone (RAA) axis, which in turn leads to renal sodium retention. However, oedema formation in this model does not require renal salt and water retention. Instead, the process of loss of fluid into the interstitium that produces oedema also caused the activation of the RAA system, which in turn led to 'secondary' renal sodium retention. Continued sodium retention is caused by the inability of increased lymphatic flow to return sufficient fluid to the plasma compartment to support a renal plasma volume, so that the additional salt and water retained by the kidney fail to suppress the RAA axis and merely contribute to further oedema formation. This produces a positive feedback loop promoting a high plasma renin activity, reduced urinary sodium excretion, increased serum and urine aldosterone concentration, and reduced plasma
FIGURE 5
MECHANISMS INVOLVED IN OEDEMA FORMATION BY 
SODIUM AND WATER RETENTION

Renal Ischemia

Decreased Na\(^+\) in renal tubules

Angiotensinogen (renin-substrate)

Angiotensin I

Angiotensin II

Aldosterone

Increase in Na\(^+\) reabsorption

Hypoperfusion

Baroreceptors

Vasomotor centre

Sympathetic outflow

Renal ischemia

Decreased GFR

Decreased excretion of Na\(^+\)

Increased renal retention of Na\(^+\) and water

OEDEMA

Increase in ADH

Increased retention of water

Source: Rai and Fernades, 1999
Note: On left and middle are the sequence of events in extra-renal hormonal and intrinsic-renal mechanisms respectively for Na\(^+\) and water retention, whereas on right is shown the ADH mechanism for water retention.
and blood volumes. Many patients with nephrotic syndrome behave exactly as predicted by this model [Kaysen, 1986]. Thus, some patients, especially those with MCNS, demonstrate high plasma renin activity and high serum aldosterone concentrations are intensely anti-natriuretic.

There are some safety factors that are developed before oedema develops and are following:

- **Negative interstitial fluid pressure**
  The interstitial pressure normally is –7mmHg. And oedema does not appear until this pressure exceeds zero.

- **Flow of lymph from the tissue increases**
  This process removes a part of the excess interstitial fluid. The increased lymph flow removes some protein from the interstitial tissues, resulting in a fall in the colloid osmotic pressure of the interstitial compartment. This is an additional safety factor.

(c )**Tissue factor**

The forces acting in the interstitial space – oncotic pressure of the interstitial space and tissue tension, are normally quite small and insignificant to counteract the effects of the plasma oncotic pressure and the capillary hydrostatic pressure, respectively. However, on some situations, the tissue factors in combination with the other mechanisms play a role in the causation of oedema. These are (i) Elevation of oncotic pressure of interstitial fluid that occurs due to increased vascular permeability and inadequate removal of proteins by lymphatics and (ii) Lowered tissue tension as seen in loose subcutaneous tissues of the eyelids and external genitalia.

**HYPERLIPIDAEMIA**

Hyperlipidaemia so commonly complicates heavy proteinuria that it has come
FIGURE 6
PRIMARY OEDEMA FORMATION WITH RESULTANT
RENAL SODIUM RETENTION

RENAL DISEASE

INCREASED GLOMERULAR PERMEABILITY TO MACROMOLECULES

LOSS OF ALBUMIN IN URINE

HYPOALBUMINAEMIA

NET LOSS OF PLASMA WATER INTO THE INTERSTITIAL SPACE

OEDEMA FORMATION

PLASMA VOLUME CONTRACTION

ACTIVATION OF RENIN ANGIOTENSIN ALDOSTERONE AXIS

RENAL SODIUM AND WATER RETENTION

RESTORATION OF PLASMA VOLUME TOWARD NORMAL
to be regarded as an integral feature of nephrotic syndrome. An association between lipids and kidney disease was first noted by Virchow, who described ‘fatty degeneration’ of the renal epithelium in Bright’s disease in 1960 [Virchow, 1960]. It has long been recognised as a frequent metabolic abnormality in nephrotic patients [Epstein, 1971]. Ever since the possible adverse effects of dyslipidaemia complicating the renal disease have attracted the interests of various investigators. Both qualitative and quantitative abnormalities in the lipid profiles have been observed in the patients with nephrotic syndrome.

Many lipid and lipoprotein abnormalities that are encountered in nephrotic patients are outlined in the table 3. Increased plasma total cholesterol concentration is the most common abnormality noticed in nephrotic patients. Plasma triglyceride levels may also be elevated, especially in the patients with heavy proteinuria [Wheeler and Bernard, 1994; Appel et al., 1985; Muls et al., 1985; Ohta and Matsuda, 1981; Gherardi et al., 1977]. Increase in the levels of phospholipids has also been described in nephrotic plasma and, although the free fatty acids concentrations are normal, the proportion bound to protein is reduced [Oetliker et al., 1980]. In general, the magnitude of lipid abnormality correlates with the severity of the disease [Wheeler and Bernard, 1994].

QUANTITATIVE PLASMA LIPID ABNORMALITIES

An increase in the number of LDL-C is virtually always present in nephrotic syndrome. The concentrations of VLDL-C are often elevated as well in these patients [Warwick et al., 1990; Joven et al., 1990; Querfeld et al., 1988; Muls et al., 1985; Gherardi et al., 1977]. Hyperchylomicronaemia has also been documented in some studies [Newmark et al., 1975]. The concentration of HDL-C has been reported to be variable [Querfeld, 1999] i.e. these concentration have either been reported as high, normal or low [Wheeler and Bernard, 1994]. Mani et al. (1988) observed decreased serum levels of HDL-C in nephrotic patients owing to the reason of its being lost
### TABLE 3
LIPID, LIPOPROTEIN AND APOLIPOPROTEIN ABNORMALITIES IN NEPHROTIC SYNDROME

<table>
<thead>
<tr>
<th>LIPID MOEITY</th>
<th>INCREASED</th>
<th>DECREASED</th>
<th>UNCHANGED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma lipids</td>
<td>Cholesterol</td>
<td></td>
<td>Free fatty acids</td>
</tr>
<tr>
<td></td>
<td>Triglyceride</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Phospholipid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipoproteins</td>
<td>LDL-C</td>
<td>HDL\textsubscript{2}</td>
<td></td>
</tr>
<tr>
<td></td>
<td>VLDL-C</td>
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<td></td>
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<tr>
<td></td>
<td>HDL-C</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(especially HDL\textsubscript{3})</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>LP(a)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apolipoproteins</td>
<td>Apo B</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Apo CII</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Apo E</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Apo CIII/CII ratio</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Apo AI*</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Apo AII</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Source: Wheeler and Bernard, 1994

*In some studies (Tesar et al., 1996) it has been found to be increased.
through the urine of these patients. In the HDL-C subfractions, a reduction in
the HDL\(_2\) and an increase in the HDL\(_3\) subfractions have been observed due
to the reduction in the lecithin:cholesterol acyltransferase (LCAT) activity
[Wheeler and Bernard, 1994]. This pattern of HDL-C disturbance, along with
increased LDL-C: HDL-C ratios [Warwick \textit{et al.}, 1990; Querfeld \textit{et al.}, 1988;
Joven \textit{et al.}, 1987 Muls \textit{et al.}, 1985] and elevated Lp(a) levels [Yang \textit{et al.},
1998], might enhance the risk of atherosclerosis in nephrotic patients
[Wheeler and Bernard, 1994]. These differences are probably due to failure to
control for the confounding variables such as therapy, inappropriate controls
and different levels of renal function. In recent studies using age and gender
matched individuals, the HDL concentrations in adult nephrotics without renal
failure or other illnesses were normal [Joven \textit{et al.}, 1990; Short \textit{et al.}, 1986;
Appel \textit{et al.}, 1985]. Irrespective of the changes in the total HDL
concentrations, the subclasses of this lipoprotein appear to be abnormally
distributed, with a reduction in the HDL\(_2\) and an increase in the HDL\(_3\) [Short \textit{et
al.}, 1986; Muls \textit{et al.}, 1985; Gherardi \textit{et al.}, 1977]. This pattern of HDL
disturbance, in conjunction with increased VLDL-C and LDL-C levels, is
associated with an increased risk of atherosclerosis [Silva \textit{et al.}, 2002;
D'Amico and Gentile, 1993; Olbricht, 1991; Miller \textit{et al.}, 1981; Lewis \textit{et al.},
1974]. In addition, since HDL\(_2\) is involved in the recycling of apo CII to VLDL
and chylomicrons (Figure 7) therefore, abnormal metabolism of these
lipoprotein classes may be interrelated [Schaefer and Levy, 1985]. A number
of recent studies have reported that Lp (a) levels are elevated in patients with
proteinuria and represent yet another risk factor for atherosclerotic vascular
disease in these patients [Short \textit{et al.}, 1992; Thomas \textit{et al.}, 1992; Kapelrud \textit{et
al.}, 1991; Karadi \textit{et al.}, 1989]. Apolipoprotein (apo) abnormalities in nephrotic
patients generally reflect changes in the lipoprotein concentrations. Thus,
plasma levels of apo B, C, and E are elevated in keeping with an increase in
the number of circulating LDL and VLDL particles, while apo AI and AII are
either normal [Joven \textit{et al.}, 1990; Ohta and Matsuda, 1981; Kashyap \textit{et al.},
1980] or found to be elevated [Tesar \textit{et al.}, 1996] in some studies.
FIGURE 7
NORMAL LIPOPROTEIN METABOLISM AND PROPOSED ABNORMALITIES IN NEPHROTIC SYNDROME

Source: Wheeler and Bernard, 1994
The concentration of apo-B, the major LDL-C protein, is generally increased proportionately more than the other lipoproteins [Joven et al., 1990], consistent with the pattern of lipoprotein abnormality [Joven et al., 1990]. Although the plasma levels of both apo CII and CIII subclasses may be elevated, the ratio of CIII to CII is increased [Short et al., 1986; Kashyap et al., 1980]. Since apo CII activates and apo CIII competitively inhibits lipoprotein lipase (LPL), such changes may contribute to defective activity of this enzyme and thus reduce lipoprotein catabolism during nephrosis [Brown and Baginsky, 1972].

QUALITATIVE PLASMA LIPID ABNORMALITIES

Some studies have suggested that unlike the primary hyperlipidaemias, qualitative abnormalities in the composition of these lipoprotein particles are present in nephrotic syndrome [Muls et al., 1985]. These abnormalities described in nephrotics include a higher ratio of cholesterol to triglyceride in apo B-containing particles and an increase in the proportion of cholesterol, cholesterol ester and phospholipid relative to protein [Gherardi et al., 1977; Muls et al., 1985]. The accumulation of these abnormal lipoproteins implies defective catabolism and has been associated with an increased risk for atherosclerosis.

PROPOSED MECHANISM OF HYPERLIPIDAEMIA

The exact mechanisms responsible for the elevated lipid concentrations in nephrotic syndrome are not fully understood [Majumdar and Wheeler, 2000]. Moreover, the signal to which the liver responds with enhanced lipid and lipoprotein synthesis remains unclear. However, various theories have been postulated/proposed and are following:

1. Increased hepatic lipoprotein synthesis The abnormal glomerular permeability causing increased permeability of plasma proteins and diminished plasma oncotic pressure may contribute to the enhanced hepatic synthesis of albumin along with the other proteins, cholesterol, triglyceride
might be enhanced in parallel with the apolipoproteins was postulated by Marsh and Drabkin (1960). This would lead to increased hepatic secretion of lipoprotein particles [Marsh and Drabkin, 1960]. In vitro studies using isolated perfused rat livers and liver slices from nephrotic rats later confirmed that the incorporation of amino acids and lipid precursors was enhanced and that lipoprotein secretion was increased [Brenner and Shafrir, 1980; Marsh and Sparks, 1979]. Increased synthesis of lipids and apolipoproteins has also been documented by in vivo studies in nephrotic rats, while the liver of these animals demonstrated cholesterol enrichment and increased activity of 3-hydroxy, 3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol biosynthesis [Marsh and Sparks, 1979; Brenner and Shafrir, 1980; Goldberg et al., 1982; Gherardi et al., 1980]. In humans with nephrotic syndrome, the turnover studies using radio-labelled glycerol and mevalonate have also shown an increase in the synthesis of both triglyceride and cholesterol. Furthermore, by trace-labelling the apo B moiety of VLDL-C and LDL-C, it has been possible to demonstrate higher turnover and increased absolute catabolic rates of these lipoproteins, which implies that their rate of synthesis is increased [Moorhead et al., 1989; Warwick et al., 1990; Warwick et al., 1991]. The data of the above mentioned studies provide strong evidence in favour of enhanced hepatic synthesis of VLDL-C in nephrotic syndrome.

The precise stimulus for enhanced hepatic lipoprotein synthesis in nephrotic syndrome is still unknown [Wheeler and Bernard, 1994]. It may be related directly to hypoalbuminaemia, because in both; nephrotic patients and rats, albumin infusions normalize the plasma lipid and lipoprotein levels. However, dextran and other oncotically active macromolecules are equally effective in this regard, which suggests that decrease in the plasma oncotic pressure, rather than hypoalbuminaemia per se, may be a more important trigger for increased lipoprotein synthesis by the liver [Majumdar and Wheeler, 2000; Appel et al., 1985]. At a cellular level, secretion of albumin, VLDL triglyceride, and apo B by cultured rat hepatocytes can be modulated by
changes in the oncotic pressure of the medium, conforming that other factors other than plasma albumin-concentration may regulate hepatic lipoprotein production [Pullinger et al., 1989; Davis et al., 1980].

It has been observed that the pharmacological agents that non-specifically reduce proteinuria have been shown to modify the plasma lipid levels without altering the rate of hepatic protein production. For example, in nephrotic rats, angiotensin-converting enzyme inhibitors lower urinary protein excretion and improve hyperlipidaemia, but do not modify the rate of albumin synthesis [Davis et al., 1990]. Such agents may also partially correct the elevated lipid levels in humans with nephrotic syndrome [Keilani et al., 1993]. These results thus suggest that urinary loss of a substance that regulates lipid metabolism may play a key role in the pathogenesis of nephrotic hyperlipidaemia.

Alternative explanations for increased hepatic lipogenesis during nephrosis have been proposed. Since the kidney is a major organ responsible for the metabolism of mevalonate (a precursor of cholesterol synthesis), impairment in the renal mevalonate metabolism may lead to elevated plasma levels and increased hepatic production of cholesterol [Golper and Swartz, 1982; Raskin and Siperstein, 1974]. In a series of experimental studies using isolated perfused rat organs, it was shown that kidneys from nephrotic animals remove less mevalonate from the perfusate than in control subjects (although the livers of these experimental rats incorporate twice as much infused precursor into cholesterol). Increased hepatic cholesterol concentrations may both enhance VLDL-C production and decrease LDL-C receptors, thereby reducing the rate of cholesterol clearance from the circulation [Brown and Goldstein, 1985].

Finally, the increased hepatic production of proteins other than lipoproteins may contribute to hyperlipidaemia during nephrosis. For example, plasma levels of cholesteryl ester transfer protein (CETP) are elevated in patients with heavy proteinuria as a result of over synthesis by the liver. This
protein mediates the transfer of esterified cholesterol from HDL to triglyceride-rich lipoproteins (Figure 7). Based on these observation that a genetic deficiency of CEPT results in reduced VLDL-C and LDL-C cholesteryl ester concentrations, it has been proposed that elevated levels in nephrotic plasma may lead to cholesterol enrichment of these lipoproteins [Moulin et al., 1992].

To summarise, the hepatic lipoprotein production is increased during nephrosis and is a major factor in the development of lipid abnormalities. Moreover, the exact stimulus or stimuli to increased lipoprotein synthesis remain to be elucidated.

2. Defective/ impaired lipoprotein catabolism

The defective or impaired lipoprotein catabolism occurs in these patients when there is some defect in any of the important enzymes that carry out important functions of lipid catabolism. Some of the enzymes are discussed below:

a) Lipoprotein lipase (LPL)

Delayed clearance of radio-labelled lipids or autologus lipoproteins and abnormal fat-tolerance tests have been described by a number of investigators both in nephrotic animal and human studies, and there seems little doubt that the lipoprotein catabolism is impaired during nephrosis [Warwick et al. 1991; Vega and Grundy 1988; Chan et al. 1981]. Measurements of LPL activity have demonstrated impaired enzyme function, and this has been presumed to be a major cause of the catabolic defect. The factors responsible for the reduced LPL activity in nephrotic syndrome have not been defined. But one possibility is that the enzyme is simply lost in the nephrotic urine; however, this has not been substantiated. Alternatively, since albumin itself augments LPL by binding free fatty acids, a product of lipoprotein hydrolysis, hypoalbuminaemia may result in free fatty acid accumulation, which inhibits the enzyme activity. [Eckel, 1989]. Finally, the urinary loss of a LPL cofactor may contribute to reduced plasma activity of the enzyme.
Candidate molecules that may influence LPL activity and that are small enough to be lost in nephrotic urine include the enzyme cofactor apo CII, HDL-C particles, which apo CII to VLDL-C and glycosaminoglycans, that tether the enzyme to the vascular endothelium. Since urinary loss of heparan sulfate, the major glycoprotein anchoring LPL, is not increased in nephrotic syndrome and thus it seems unlikely that glycosaminoglycan deficiency alone is responsible for impaired lipoprotein catabolism. The urinary loss of HDL-C may impair the recycling of apo CII to VLDL-C and thereby reduce LPL activity. HDL-C is only slightly larger than albumin and is the predominant lipoprotein particle found both in rat and human urine [Shafrir et al., 1990; Mani et al., 1988; Saku et al., 1988; Short et al., 1986; Gherardi et al., 1980; de Mendoza et al., 1976; Feits and Mayerle, 1974]. The quantitative estimates of HDL-C excretion have ranged from less than 1% to greater than 50% of the total circulating lipoprotein particles daily in the patients with urinary protein excretion in the range of 6 to 10g/24h [Joven et al., 1990; Short et al., 1986].

b) Lecithin cholesterol acyl transferase (LCAT)

Several studies suggest that factors other than reduced LPL activity are responsible for impaired lipoprotein catabolism in nephrosis [Wheeler and Bernard, 1994]. The reduced activity of another key enzyme in lipoprotein catabolism, which is LCAT, has also been documented in rats and human nephrotic plasma. Hypoalbuminaemia may again be responsible for this defect, since albumin normally binds lysolecithin, one product of the LCAT reaction, and accumulation of lysolecithin impairs the activity this enzyme [Sestak et al., 1989; Cohen et al., 1980]. Alternatively, LCAT may be depleted as a result of its loss in urine [Gherardi et al., 1980]. Low LCAT levels would impair cholesterol esterification within the HDL-C particle, thereby inhibiting the conversion of HDL₃ to HDL₂. This defect in the HDL-C maturation would in turn reduce the transfer of apo-CII to VLDL-C and may thus inhibit catabolism of triglyceride-rich lipoprotein [Eisenberg, 1984; Cohen et al., 1980], defects that could explain many of the lipoprotein abnormalities associated with nephrotic syndrome.
c) Cholesterol ester transfer protein (CETP)

Studies in humans with nephrotic syndrome have reported the levels of CETP to be increased [Moulin et al, 1992; Sparks et al, 1981; Braschi et al, 1997]. In addition to the high mass levels of the protein, its specific activity is enhanced by the increased concentration of free fatty acids in the nephrotic lipoproteins. The implications of high levels of CEPT are two-fold – First, the protein is synthesised by the liver, conforming to the observation that many of the plasma proteins secreted by the liver are increased in nephrotic plasma. Second, normal or increased activity of LCAT followed by transfer of cholesterol ester from HDL to VLDL as well as to LDL would exacerbate the pro-atherogenic dyslipidaemia. (In addition, this would initially increase the TG content of HDL-C, which favours the increased catabolism).

3. Defective receptor clearance of lipoprotein particles

A further abnormality that may contribute to nephrotic hyperlipidaemia is the defective removal of IDL-C and LDL-C from the circulation via lipoprotein receptors (Figure 7). A study carried out by Warwick et al, 1990 reported a 55% reduction in the receptor-mediated clearance of LDL-C and an increased catabolism via alternative pathways. But a subsequent study by the same investigators and other investigators [Joven, 1990] showed only a non-significant trend towards a decrease in the LDL-C catabolism. Other investigators have failed to find a difference in the lipoprotein clearance in the nephrotics compared with controls [Gitlin et al 1958; Scott et al, 1970]. In addition to the methodological differences, the inclusion of patients with uremia and ex-vivo oxidation of LDL-C samples may account for these conflicting results. While further kinetic studies may resolve these differences, in vitro evaluation of lipoprotein-receptor difference may be a more useful way to establish whether cellular uptake of LDL-C is truly impaired in NS [Wheeler and Bernard, 1994].
4. Increased urinary excretion of HDL-C / lipiduria:

As the duration and amount of albuminuria increases, the urinary excretion of HDL-C [Mani et al, 1988] increases in these patients. This pattern of HDL-C disturbance, along with increased LDL-C:HDL-C ratio might enhance the risk of atherosclerosis during nephrosis. To summarise, although there is good evidence to suggest that the defective catabolism of triglyceride-rich lipoproteins contribute to nephrotic hyperlipidemia and that reduced activity of catabolic enzymes seems to play a role in this and the specified factors responsible have not been clearly established.

In addition, to the above proposed mechanisms, an early hypothesis of the origin of the nephrotic hyperlipidaemia was that of Marsh and Drabkin (1960). They postulated that proteinuria led to overproduction of hepatic secretary proteins, especially albumin, and including lipoproteins. The lipoproteins that are too lager to enter the glomerular filtrate even in the presence of glomerular damage, would then continue to rise in the plasma. Some urinary loss of LDL-C is occasionally observed and almost all of the urinary lipoprotein is HDL-C that is modified during its passage through the kidney. These observations apply both in humans [Shore et al, 1982] and rats [Marsh et al, 1996].

For further understanding of the lipoprotein abnormality observed in these patients, the metabolism of lipoprotein fractions in nephrotic patients will be discussed below:

A. Metabolism of VLDL-C

Increased hepatic synthesis of VLDL apo B, the critical structural apolipoprotein of VLDL-C and LDL-C has been demonstrated in nephrotic rats [Marsh, 1984]. In addition, decreased catabolism of VLDL-C has also been consistently observed [Marsh, 1996]. In nephrotic syndrome, the metabolism
of VLDL apo B has been examined in three recent studies using amino acid precursors labelled with isotopes. This approach avoids potential artefacts in earlier work employing re-injection of the subject's VLDL-C after *in vitro* radioiodination.

Aguilar-salinas *et al* [1995] studied four subjects with nephrotic range proteinuria (>3.5g/day) and found a significant 59% decrease in the fractional catabolic rate (FCR) of VLDL apo B100. Though the average production rate (PR) increased by 62%, this increase was not statistically significant (p=0.08). Three of four subjects had elevated TG levels. In a study of seven subjects, Demant *et al* [1998] found that the increased plasma concentration of VLDL-C was due to decreased catabolism. Their subject's had average TG levels twice than that of the controls. They found no correlation with the rate of albumin synthesis and so concluded that a general increase in hepatic protein synthesis is not the primary cause of nephrotic hyperlipidaemia. De Sain-van der Velden *et al*, [1998] in a similar study of six subjects with elevated TG levels, found a decreased FCR of VLDL apo B100 with no significant increase in the PR. There seems little doubt from the studies and from earlier work with radioiodinated VLDL [Marsh *et al*, 2002] that in humans with established nephrotic syndrome, VLDL apo B100 levels, as well as plasma TG levels are elevated because of decreased catabolism. An increased PR, though seen in some subjects, is not a consistent finding.

Once hyperlipidaemia is established, the apolipoprotein composition of VLDL-C changes. Deighan *et al* [2000] reported that large VLDL-C particles were deficient in apo CII, apo CIII and apo E. They indicated that these changes were associated with increased free cholesterol to phospholipid ratio and a smaller average particle size. The smaller VLDL-C particles were associated with a decreased apo E content. Since apo CII is an essential activator of lipoprotein lipase, and apo CIII inhibits the enzyme, these changes can explain decreased lipolysis independent of the lipolytic enzyme activity and decreased VLDL-C catabolism. Decreased apo E could contribute to the
elevation of IDL-C. Altered apolipoprotein composition could also affect receptor-mediated uptake of VLDL-C. In nephrotic rats, a deceased expression of the VLDL receptor has been shown which is responsible for uptake of intact VLDL-C by muscle and adipose tissue [Liang and Vaziri, 1997].

B. Metabolism of LDL-C

In human nephrotic syndrome, increased production of LDL apo B100 has been found in several studies, (the metabolism of LDL can be studied by labelling its apo B100, either by radio-labelling in vitro or by endogenous labelling with an amino acid) but there has been some inconsistency in this finding. Earlier studies by Joven et al [1990] using radio-labelled LDL-C concluded that an increased PR, and not a decreased FCR, raised the LDL-C levels. Warwick et al [1990] reached the opposite conclusion. A study by Vega et al [1995] pointed to the TG level as an important variable that correlated with the production of LDL apo B100. They found that subjects overproduced LDL-C only when elevated TG as well as elevated cholesterol levels were observed, whereas subjects showing only hypercholesterolaemia had a decreased rate of clearance (FCR).

In the more recent studies, mainly using stable isotopes, an increased PR of LDL apo B100 has also been observed. In 4 subjects compared to 4 controls, the PR was increased, with no increase in the transfer of apo B100 from VLDL-C to LDL-C [Aguilar-Salinas et al, 1995]. In another study of 7 subjects compared to 8 controls, a marked increase in the PR was found, with no decrease in the FCR [De sain-van Velden et al, 1998]. In a study of 7 nephrotic subjects, Stenvinkel et al [1997] observed a significant two-fold increase in the LDL apo B100 production and normal FCR, compared to 41 controls. The elevated PR was inversely correlated with the plasma albumin level (r = -0.82). Demant et al [1998] noticed in 7 subjects that the LDL-C catabolism was significantly reduced with only a trend towards an increased PR. It seems reasonable to conclude that hepatic overproduction of LDL apo
B100 is a critical event in establishing the hypercholesterolaemia of the nephrotic syndrome.

C. Metabolism of HDL-C and apo A1

The levels of HDL-C and apo A1 are reported to be higher in rats while in humans serum levels of HDL-C have been reported to be either increased, decreased or normal. In rats (expressing the human apo A1 gene), with PAN-nephrosis resulted in an increase in plasma levels of apo A1 reaching 10mg/ml in spite of urinary losses. In experimental nephrosis, expanded pool of HDL-C leads to a saturation of the processes involved in HDL-C catabolism, resulting in a decreased FCR [Kaysen et al, 1995; Sparks et al, 1981].

CONSEQUENCES OF HYPERLIPIDAEMIA IN NEPHROTIC SYNDROME

Hyperlipidaemia during nephrosis leads to the following consequences

A] Cardiovascular disease

Hypercholesterolaemia and the metabolic cluster of hypertriglyceridaemia associated with low HDL-C levels are proven risk factors for ischaemic heart disease in the general population. Moreover, many of these lipid abnormalities associated with nephrotic syndrome, notably increased TC, LDL-C, VLDL-C, reduced HDL$_2$ relative to HDL$_3$, increased apo-B and Lp (a) concentrations have also been associated with an increased risk of cardiovascular disease. A recent retrospective analysis of 142 nephrotics showed that after corrections had been made for hypertension and smoking habits, the relative risk of myocardial infarction was increased 5.5 fold compared with the controls. In 129 haemodialysis patients who were prospectively followed for four years, demonstrated that Lp (a) levels were an independent risk factor for cardiovascular events. Due to the direct correlation between serum albumin, total cholesterol and apo-B in these patients, this was interpreted as reflecting a severe co-morbid illness of those with the highest chance of dying [Wheeler and Bernard, 1994]. Isolated necropsy
reports of premature coronary atherosclerosis in young patients with nephrotic syndrome, only one study has attempted to determine whether the incidence of coronary artery lesions is greater than in age-matched controls [Silva et al, 2002]. To establish whether correction of the lipid abnormalities will reduce the risk of ischemic heart disease, or other cardiovascular complications, in nephrotic patients requires controlled interventional studies of lipid-lowering agents (natural or pharmacological). This risk could be very high. The patients with familial hypercholesterolaemia, who have plasma cholesterol levels comparable to individuals with nephrotic syndrome, experience a rate of coronary artery disease of 20% at age 40 and 75% at age 60 [Olbricht and Koch, 1992]. With the evidence currently available, it seems reasonable to conclude that hyperlipidaemia during nephrosis represents a serious risk factor for progressive atherosclerosis, at least in some patients [Wheeler and Bernard, 1994].

B) Progressive renal disease

Recent experimental evidence supports the hypothesis that lipids contribute directly to glomerulosclerosis and tubulointerstitial injury and that correction of lipid abnormalities associated with renal disease will slow the progression of chronic renal failure [Majumdar and Wheeler, 1994; Wheeler and Bernard, 1994; Keane et al, 1991]. Histological and histochemical studies in animals have shown deposits containing cholesterol, triglycerides, phospholipids, apolipoproteins (apo-A, B and E), oxidised LDL-C particles in the glomeruli [Anderson et al, 1987]. Since, the normal glomerular capillary is lined by a fenestrated endothelium (podocyte), macromolecules as large as lipoproteins may gain relatively unimpeded access to the mesangium, but are usually returned to the capillary via a similar route/ cleared via lymphatic channel. Mesangial deposition of lipoproteins may occur when these clearance mechanisms are defective or overloaded following loss of functional nephron mass. The lipid deposition in the glomerulus promotes mononuclear cell infiltration [O'Meara and Brady, 1997], another recognised feature of early glomerulosclerosis. Monocytes that infiltrate the mesangium differentiate into
tissue macrophages that ingest deposited lipids to become foam cells. These cells may then release inflammatory mediators that modify glomerular function. In vitro studies suggest lipoproteins may act in concert with these mediators to promote glomerular injury. Since LDL-C stimulates production of monocyte chemo-attractant protein-1 by mesangial cells in vitro, this seems a likely mechanism by which LDL-C mediates the inflammatory response. These lipid depositions as discussed earlier result in the release of the inflammatory mediators, in addition also lead to disturbed secretory function of the mesangial cell, death of mesangial cell and excess accumulation of matrix components eventually leading to irreversible scarring [Majumdar and Wheeler, 2000]. These events are similar to those thought to transform fatty streaks into fibrous plaques in the arterial wall. In adults and children with non-diabetic renal disease, elevated plasma lipid levels have been associated with faster rate of deterioration of renal function. A positive relationship between hyperlipidaemia and the rate of progression of renal damage suggests that lipids can induce/aggravate glomerular injury mainly by interacting with mesangial cells, and also induce endothelial cell dysfunction. Fuiano et al (1996) also concluded that the reduction in cholesterol is associated with a significant increase in renal plasma flow, thus suggesting that hypercholesterolaemia may actually impair the renal hemodynamics. It was speculated that this effect might contribute to increase in the risk of ischemic acute renal failure during nephrosis and to the progression of renal disease. In another study carried out by Tsukahara et al [1997] reported that children with frequently relapsing nephrotic syndrome have prolonged periods of hypercholesterolaemia even during clinical remission and they require careful monitoring of the serum lipid profiles.

The alterations in lipoprotein metabolism in renal disease resulting in the elevated levels of apo-B containing lipoprotein may reflect in hyperlipidaemia. Apolipoprotein B containing lipoproteins occur in VLDL-C, IDL-C and LDL-C. Various authors [Vega 1995; Krammer et al, 1994; Ongajyooth 1993; Attman 1990] have concluded that an increased
concentration of apolipoprotein-B is observed in these patients. Hyperlipidaemia and heavy proteinuria during nephrotic syndrome is associated with increased formation of cholesterol-rich apo-B-containing lipoprotein in LDL-C and VLDL-C. The characteristic feature in renal failure is the accumulation of intact or partially metabolised triglyceride rich lipoprotein in IDL-C and VLDL-C. The potentially atherogenic apo-B containing lipoproteins have been linked to pathogenic processes that result in progressive glomerular and interstitial lesions and ultimately leading to the loss of renal function [Attman, 1997]. The mechanism of injury is not fully understood. Nevertheless, one explanation is that the receptor and non-receptor mediated uptake of lipoproteins by mesangial cells may induce / accelerate proliferative and sclerotic processes in glomerular mesangium that are analogous to atherosclerosis in the arterial wall. Changes in the glomerular permeability can result in increased filtration of lipoproteins that may be internalised by tubular cells and illicit corresponding lesions in the interstitial tissues. The negative impact of proteinuria on the prognosis of renal disease could be mediated in part through an increased filtration of lipoproteins [Mani and Mani, 1989]. Induction of hyperlipidaemia, which is a common feature of nephrotic syndrome, is thought to promote the progression of glomerular injury. It is believed that the cholesterol enrichment of VLDL-remnant and LDL-C is presumably caused by enhanced cholesterol-ester transfer to these lipoproteins that indicate prolonged persistence of lipoproteins in the circulation. Accumulating particles penetrate the vascular endothelium [Nielsen et al., 1997], undergo oxidative modification in the sub-endothelial space and may associate with the matrix components (Figure 8). Apo B and E containing lipoproteins that are modified by lipid peroxidation were detected along with immune deposits at the GBM in passive Heymann nephritis [Exner et al., 1996]. Within the GBM considerable amounts of hydrogen peroxide were detected in the same model of experimental membranous glomerulonephritis [Neale et al., 1993]. Similar to the deposition of oxidised LDL-C in a focal and segmental [Mugge et al., 1994; Ohara et al.,
FIGURE 8

SCHEMATIC REPRESENTATION OF THE ARTERIAL WALL IN THE EARLY PHASE OF ARTERIOSCLEROSIS

1993], which lead to defective endothelial distribution pattern was seen in the glomeruli of rats with focal and segmental glomerulosclerosis [Lee et al., 1997]. A study was carried out by Wanner and co workers [1997] to assess whether oxidised LDL and lipoprotein (a) [Lp (a)] influence the following three major systems: (i) endothelium-dependent vasodilation, (ii) renin release of juxtaglomerular (JG) cells and (iii) proliferation and viability of mesangial cells (MC). These major systems are discussed slightly in detail below.

(i) Endothelial dysfunction in renal arteries

Oxidised LDL has been shown to interfere with the formation of nitric oxide (NO) and also to directly inactivated nitric oxide [Chin et al., 1992; Galle et al., 1991; Tanner et al., 1991]. Thus, interference of atherogenic lipoproteins with nitric oxide in renal arteries could increase vascular tone and reduce renal blood flow. The data of Wanner and co workers (1997) indeed demonstrates that oxidised LDL as well as Lp (a) impaired the endothelial function and attenuated the vascular tone. Results of another study suggest that the endothelial dysfunction during nephrotic syndrome may be a consequence of the increased susceptibility to oxidation, secondary to antioxidant deficiency [Posadas-Sanchez, 2001; Mosinger, 1999]. Oxidised lipoproteins significantly stimulated O$_2^*$ production in the isolated arteries [Galle et al., 1995], an effect that was prevented by anti-oxidative enzymes and HDL. Results of Wanner et al's (1997) study suggest that enhanced nitric oxide inactivation by O$_2^*$ could be the underlying mechanism for the impairment of the endothelium-dependent dilations (figure 8).

(ii) Renin release

The renin producing JG apparatus is located in close vicinity to nitric oxide producing cell such as the arterial endothelial cells, macula densa cells, or smooth muscle cells. Since nitric oxide is a potent modulator of renin secretion and the lipoproteins react with nitric oxide and inactivate its vasodilatory capacity [Chin et al., 1992; Galle et al., 1991], and so one may assume that lipoproteins also modulate the renin release. Indeed, oxidised
LDL-C and Lp(a) directly stimulate the renin release from the isolated JG cells in the primary culture [Galle et al., 1995]. Experiments with O$_2^-$ and H$_2$O$_2^-$ metabolising enzymes (superoxide dismutase and catalase) indicate that the stimulation of renin release by oxidised LDL-C and Lp(a) was mediated by reactive oxygen species (ROS) [Galle et al., 1995]. When rennin is released, its stimulation might contribute to rennin-dependent hypertension and cardiovascular disease in kidney disease. The release of renin could be prevented by HDL-C via inhibition of O$_2^-$ formation (Figure 9).

(iii) Stimulation of O$_2^-$ production / oxidative damage occurring in the kidneys

Reactive oxygen species (ROS) are produced at high rates in a variety of inflammatory and non-inflammatory kidney diseases. Sources for ROS in the kidney are the resident cells, infiltrating polymorphonuclear neutrophils and other circulating cells [Ichikawa et al., 1994]. ROS are a part of the renal defence mechanisms, such as against the microbes, but their formation may also damage the renal tissue (Figure 9). Studies have shown that the formation of O$_2^-$ is enhanced in the arteries of hypercholesterolaemic animals function. Evidence [Hansen et al., 1994] in the literature suggests that the lipoprotein preferentially in their modified form may inhibit the nitric oxide mediated vasodilation in the renal arteries via stimulation of O$_2^-$ formation.

The increased risk of atherosclerosis in nephrotic syndrome is attributable in part to the associated hyperlipidaemia. The importance of oxidation of LDL in the atherogenic process has been recognised over many years now [Skrzep-Poloczek, 2001]. Based on the earlier observations of Kimmelstiel, Wilson and French, several investigators have recently gathered experimental evidence in rats and other small mammals that hyperlipoproteinaemia and hypercholesterolaemia can induce and contribute to fibrosis in the glomerular capillaries [Grone et al., 1994]. And the term 'capillary atherosclerosis' was coined by Diamond and Karnovsky [1988]. There are indications that lipid-modulated glomerular damage also occurs in humans [Grone et al., 1994]. It was observed that in patients with LCAT
FIGURE 9

SCHEMATIC REPRESENTATION OF THE ARTERIAL WALL AND THE SUBENDOTHELIAL SPACE OF AN ARTERY APPROACHING THE MACULA Densa WHERE SMOOTH MUSCLE CELLS TRANSFORM TO EPITHELOID CELL TYPES CONTAINING RENIN GRANULES.

deficiency have produced dyslipidaemia with lipid deposits in glomeruli and in consequence develop severe glomerulosclerosis. In addition, apo B and E, and LDL-receptor proteins have been detected in the glomeruli of the patients with different glomerular diseases [Takemura et al., 1993; Naito et al., 1991]. Lee and Kim [1998] reported that oxidatively modified lipoproteins could be found in human glomeruli with immune complex and degenerative diseases often associated with apo B-100. To further prove this point, the authors studied a large number of renal biopsies to demonstrate oxidatively modified lipoproteins in segmental scars and mesangium. Interestingly, they not only observed that oxidatively modified lipoproteins were found in the glomerular disease characterised by an influx of polymorphonuclear leukocytes and monocytes/macrophages (such as in membranoproliferative glomerulonephritis), but also in a rather high percent (>25%) of the patients with degenerative diseases (nephrosclerosis). Dobreanu and Mody [1997] concluded that the susceptibility of LDL-C to oxidation depends both on the concentration of pro-oxidants stimuli and also on the decreased concentration of the anti-oxidants. An observation of Warwick and co-workers [2000] suggest that there may be a relative defect in the oxidant: antioxidant balance in nephrotic patients, which could predispose to increased oxidative stress. It is also clear from figure 10 that once the oxidative stress is increased, it causes increased lipid peroxidation leading to increased glomerular damage. A study was carried on rats with chronic puromycin aminonucleoside (PA) induced nephrotic syndrome with high cholesterol diet (HC) (PA-HC). The results revealed that the susceptibility of plasma VLDL and LDL to in vitro oxidation was significantly increased. This suggested that hypercholesterolaemia could make the lipoproteins more susceptible to oxidation. But administration of vitamin E (vit E) in the rats with PA-HC resulted in significant reduction in the susceptibility of plasma VLDL and LDL to in vitro oxidation [Lee et al., 1997]. As is the case for human glomerular disease showing the deposition of apo B containing lipoproteins in the glomeruli, the intraglomerular lipid deposition has been demonstrated in rats with PA-HC by red oil staining. Increased collagen or other extracellular matrix
FIGURE 10
LIPID INDUCED GLOMERULAR INJURY

↑ Oxidative Stress

↑ LDL Cholesterol

↓ Anti-Oxidant Status

↑ Lipid Peroxidation

↑ Glomerular Cell Injury
in the diseased glomeruli together with higher circulating levels of lipoproteins may contribute to the retention of LDL-C in the mesangial matrix. When the LDL-C is trapped in the mesangial matrix with depleted antioxidants, it may be oxidised by free radical process. The free radicals produced in the rats at the time of PA delivery in the rats may cause not only podocyte injury, but also the oxidation of LDL-C in the rats with PA-HC. In addition, several in vitro studies suggest that the radicals may be delivered from the mesangial cells or infiltrating macrophages. They also demonstrated the occurrence of oxidised LDL-C (ox-LDL-C) in the glomeruli of rats with PA-HC by immunostaining, indicating that the oxidative modification of LDL-C occurs in vivo. Furthermore, administration of vit E diminished the extent/intensity of staining for ox-LDL-C. They concluded that hypercholesterolaemia in rats aggravates histologic injury in association with increased renal lipid peroxides and lipoprotein susceptibility to in vitro oxidation in rats with chronic (PAN induced) nephrosis, whereas anti-oxidants ameliorates these processes. Thus this observation suggests that a dietary anti-oxidant like vit E attenuates chronic progressive renal injury in rats with PA-HC possibly by making lipoproteins resistant to oxidation and by limiting the infiltration of macrophages in the glomeruli. Skrzep-Poloczek [2001] investigated the disturbances of the oxidant or anti-oxidant status in children with nephrotic syndrome and witnessed significant disturbances in the oxidant status these children during nephrosis. This leads to the accumulation of ox-LDL and cholesterol oxidation products in the plasma, which exert cytotoxicity and are known to induce atherosclerosis. The investigator suggests that this may constitute an important link between nephrotic syndrome and atherosclerosis. It has been observed that a high dose combination of anti-oxidant nutrients reduces the susceptibility of LDL-C to oxidation in the patients with coronary vascular disease and may be useful in secondary prevention of the disease. An animal study revealed that the antioxidant intervention in experimental high cholesterol diet reduces the LDL-C oxidation and preserves the renal vascular responses to the endothelium-dependent vasodilators. Thus, this beneficial
effect can potentially protect the kidney from hypercholesterolaemia-induced damage.

As described above, the oxidised LDL-C and Lp(a) significantly stimulated $O_2^-$ production in isolated arteries [Galle et al., 1995]. Furthermore, anti-oxidative enzymes could prevent the damaging effect of the lipoproteins on the nitric oxide-mediated vasodilation. Thus, direct and indirect evidence for the active formation of ROS in the isolated arteries after incubation with oxidised lipoproteins could be provided. These data fit well into the concept of a central role for oxidised lipoproteins in the development of endothelial dysfunction. Further support for this concept derives from human studies, where the oral supplementation of antioxidants in hypercholesterolemic patients improved their endothelial dysfunction [Anderson et al., 1995]. A study on the total antioxidant status (TAS) in nephrotic children was carried out with the aim of determining the effect of dietary antioxidants in antioxidant enzymes: Superoxide dismutase, and Glutathione peroxidase activity and on the TAS in these group of children. They concluded that reduced antioxidant protection is one of the factors leading to renal injury and may be partly associated with low intake of some vital components of the antioxidant system [Zachwieja et al., 2001; Zachwieja et al., 2000]. This can be attenuated by the supplementation of dietary supplements or natural agents containing high levels of antioxidants, anti-inflammatory properties along with hypolipidaemic properties.

From the above studies it can be derived that the atherogenic lipoproteins induce the formation of oxygen radicals not only in the arteries, but also in the glomerular and JG cells which, induce the inhibition of nitric oxide-mediated vasodilation, stimulation of rennin release, modulation of mesangial cell growth and proliferation. The anti-oxidative enzymes or antioxidant supplements and HDL-C, can prevent all the above bad effects of the atherogenic lipoproteins that induce the formation of oxygen radicals.
SPIRULINA: “NATURE’S ENCAPSULATED MIRACLE”

Health awareness among the people has grown tremendously in the past few years. With the growing knowledge and awareness about the various diseases and its preventive measures, people are now trying to prevent the occurrence of the disease by following the preventive measures (rather than to end up in the hospitals), whether it is through exercise, diet or through a food supplement (like herbal medicines and nutraceuticals). One such encapsulated nutraceutical food that is nature’s richest and most complete source of organic nutrition is ‘spirulina’.

INTRODUCTION

_Spirulina_ is a microscopic blue-green algae. It is vegetable plankton and its cells form the shape of a coiled spring (thus, the name ‘spirulina’ which means ‘little spiral’). Unlike other plants and animals, _spirulina_ does not have complicated bodies and biochemistries to maintain. Its total function is to produce proteins, carbohydrates, vitamins (like beta carotene), minerals (like selenium) amino acids, protective pigments, anti-inflammatory enzymes like superoxide dismutase and many other vital nutrients important in human health (like γ-linolenic acid) [www.spirulina.com].

HISTORICAL ASPECTS

It is believed that the algae were the first form of life on earth. Scientists have discovered fossils of algae structurally resembling _spirulina_, which dates back to about 3.5 billion years. There is description of microalgae in the Holy Bible too. The Bible describes it as "when the people of Israel were starving in the wild, God provided them 'MANNA', which they collected and baked it into bread". 'MANNA' is a kind of lichen i.e. combination of fungus and blue-green algae which usually grows on the rock crests. The Aztecs, native Mexicans living near the Lake of Texcoco called the algae as a ‘sacred power plant’ owing to its property of providing high energy.

In the post World War II era, there was a worldwide alarm concerning the global protein shortage. According to the Food and Agriculture
Organisation report, 16% of the world population suffered from severe undernutrition and 30-50% suffered from malnutrition. To overcome the protein malnutrition problem, action was taken to explore alternative protein source other than the conventional cereal protein.

Dr. Christopher Hills, a young scientist from England, working with Dr. Hiroshi Nakamaru, "re-discovered" spirulina and its biological superiority and became known as the "Father of Spirulina". In the past two decades, spirulina has reintroduced itself to humanity and has gained worldwide acceptance. It is also gaining more attention from the medical scientists as a nutraceutical and source of potential pharmaceutical. Today, scientists at the National Aeronautics and Space Administration are trying to grow spirulina at the space stations, to convert carbon dioxide exhaust of the space stations to oxygen, and are increasingly being used as the 'space age food'. In short, spirulina contains billions of years of successful evolutionary wisdom coded in its deoxyribose nucleic acid.

**NUTRITIONAL COMPOSITION OF SPRAY-DRIED SPIRULINA**

Its physical and nutritional composition is depicted in table 4. The cell wall of spirulina consists of mucopolysaccharide and not hard cellulose that is present in most of the plants; therefore, it can be easily digested and assimilated in the body. The soft cell wall of spirulina is made up of about 16% of carbohydrate and sugars. Glycogen and rhamnose, two polysaccharides are easily absorbed by the body. Glycogen is readily converted by the body to glucose and is utilised for the production of energy that is needed for body processes. Rhamnose is a rare and biologically active sugar that transports nutrients across the blood brain barrier. Highest protein content of spirulina (65%) among all natural foods like soybean (35%), fish (25%), dehydrated milk (35%), eggs (12%), gains (8-14%) or peanuts (25%). Spirulina also contains natural bio-chelated vitamins (in highly bioavailable form).

Spirulina has emerged as a great diet phenomenon, through innovative research. The various health benefits of spirulina have been listed in table 5.
TABLE 4
PHYSICAL PROPERTIES AND NUTRITIONAL COMPOSITION OF SPIRULINA

Physical properties
1) Appearance – Fine powder  
2) Colour – dark blue-green  
3) Particle size – 64 mesh through  
3) Odour and taste—mild like seaweed

Nutritional Composition of spray dried *spirulina* powder

<table>
<thead>
<tr>
<th>S. No</th>
<th>Nutrients</th>
<th>Quantity (per 100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PROXIMAL PRINCIPLES</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Moisture</td>
<td>6.72%</td>
</tr>
<tr>
<td>2</td>
<td>Ash</td>
<td>5.89%</td>
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<tr>
<td>3</td>
<td>Crude fibre</td>
<td>9.34%</td>
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<tr>
<td>4</td>
<td>Carbohydrates</td>
<td>16.05%</td>
</tr>
<tr>
<td>5</td>
<td>Total protein</td>
<td>64.63%</td>
</tr>
<tr>
<td>6</td>
<td>Fat</td>
<td>6.71%</td>
</tr>
<tr>
<td>7</td>
<td>Calories</td>
<td>346Kcal</td>
</tr>
<tr>
<td></td>
<td>VITAMINS</td>
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</tr>
<tr>
<td>1</td>
<td>β-Carotene</td>
<td>320.000IU</td>
</tr>
<tr>
<td>2</td>
<td>Biotin</td>
<td>0.22mg</td>
</tr>
<tr>
<td>3</td>
<td>Cyanocobalamin (B₁₂)</td>
<td>65.7μg</td>
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<tr>
<td>4</td>
<td>Folic acid</td>
<td>17.6μg</td>
</tr>
<tr>
<td>5</td>
<td>Inositol</td>
<td>0.018μg</td>
</tr>
<tr>
<td>6</td>
<td>Niacin</td>
<td>6.69μg</td>
</tr>
<tr>
<td>7</td>
<td>Pyriodoxine</td>
<td>0.39μg</td>
</tr>
<tr>
<td>8</td>
<td>Riboflavin</td>
<td>1.78mg</td>
</tr>
<tr>
<td>9</td>
<td>Thiamin</td>
<td>0.118mg</td>
</tr>
<tr>
<td>10</td>
<td>Tocopherol</td>
<td>0.733IU</td>
</tr>
<tr>
<td></td>
<td>FATTY ACID PROFILE</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>C12:0</td>
<td>12.3%</td>
</tr>
<tr>
<td>2</td>
<td>C14:0</td>
<td>1.7%</td>
</tr>
<tr>
<td>3</td>
<td>C16:0</td>
<td>34.6%</td>
</tr>
<tr>
<td>4</td>
<td>C16:1</td>
<td>8.6%</td>
</tr>
<tr>
<td>5</td>
<td>C18:0</td>
<td>0.8%</td>
</tr>
<tr>
<td>6</td>
<td>C18:1</td>
<td>3.0%</td>
</tr>
<tr>
<td>7</td>
<td>C18:2</td>
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<tr>
<td>8</td>
<td>C18:3</td>
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</tr>
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<td>Quantity (per 100g)</td>
</tr>
<tr>
<td>------</td>
<td>----------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td>IV</td>
<td>AMINO ACIDS</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Alanine</td>
<td>4.60%</td>
</tr>
<tr>
<td>2</td>
<td>Arginine</td>
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<tr>
<td>3</td>
<td>Aspartic acid</td>
<td>5.75%</td>
</tr>
<tr>
<td>4</td>
<td>Cystine</td>
<td>0.474%</td>
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<tr>
<td>5</td>
<td>Glutamic acid</td>
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<tr>
<td>6</td>
<td>Glycine</td>
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<tr>
<td>7</td>
<td>Histidine</td>
<td>1.01%</td>
</tr>
<tr>
<td>8</td>
<td>Isoleucine</td>
<td>3.69%</td>
</tr>
<tr>
<td>9</td>
<td>Leucine</td>
<td>6.18%</td>
</tr>
<tr>
<td>10</td>
<td>Lycine</td>
<td>2.99%</td>
</tr>
<tr>
<td>11</td>
<td>Methionine</td>
<td>1.38%</td>
</tr>
<tr>
<td>12</td>
<td>Phenylalanine</td>
<td>2.87%</td>
</tr>
<tr>
<td>13</td>
<td>Proline</td>
<td>2.66%</td>
</tr>
<tr>
<td>14</td>
<td>Serine</td>
<td>3.13%</td>
</tr>
<tr>
<td>15</td>
<td>Threonine</td>
<td>3.04%</td>
</tr>
<tr>
<td>16</td>
<td>Tyrosine</td>
<td>2.74%</td>
</tr>
<tr>
<td>17</td>
<td>Valine</td>
<td>4.18%</td>
</tr>
<tr>
<td>V</td>
<td>MINERALS</td>
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</tr>
<tr>
<td>1</td>
<td>Calcium</td>
<td>658mg</td>
</tr>
<tr>
<td>2</td>
<td>Phosphorus</td>
<td>977mg</td>
</tr>
<tr>
<td>3</td>
<td>Iron</td>
<td>44.7mg</td>
</tr>
<tr>
<td>4</td>
<td>Sodium</td>
<td>796mg</td>
</tr>
<tr>
<td>5</td>
<td>Chloride</td>
<td>1.14mg</td>
</tr>
<tr>
<td>6</td>
<td>Magnesium</td>
<td>445mg</td>
</tr>
<tr>
<td>7</td>
<td>Zinc</td>
<td>6.80mg</td>
</tr>
<tr>
<td>8</td>
<td>Potassium</td>
<td>1.28mg</td>
</tr>
<tr>
<td>VI</td>
<td>ENZYME</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Superoxide dismutase</td>
<td>15000IU/gm</td>
</tr>
</tbody>
</table>

* Source: New Ambadi Estate, Chennai.
TABLE 5
POTENTIAL HEALTH BENEFITS OF SPIRULINA

1. An excellent source of complete protein:
   - About 65 to 72%, easily digestible and assimilable proteins
   - All essential amino acids present
   - Highest protein content of spirulina (65%) among all natural foods like soybean (35%), fish (25%), dehydrated milk (35%), eggs (12%), gains (8-14%) or peanuts (25%).

2. Rich source of natural vitamins:
   - Natural bio-chelated vitamins, containing all the vitamins in highly bioavailable form.
   - Rich source of β-carotene, the provitamin A, several times higher than that of carrots.
   - Only vegetarian source of vitamin B₁₂ (12 times that of RDA).
   - As it contains natural vitamins, over consumption does not cause hypervitaminosis.

3. Rich source of organic minerals:
   - Over 14 minerals, including iron and magnesium, naturally balanced and chelated for assimilation.
   - Iron from spirulina is better absorbed than ferrous sulphate due to their existence in the organic form.

4. Whole food source of essential fatty acids:
   - The much needed omega 3- and 6-fatty acids.
   - Gamma-linolenic acid (GLA) (anti-inflammatory effect).

5. Others:
   - Over 2000 active enzymes, most important of them is superoxide dismutase (that has anti-inflammatory effect)
   - Contains 15-20% of starch in the form of carbohydrates like glycogen, which is easily absorbed with minimum insulin intervention.
   - Rhamnose, a rare and biologically active sugar that transports nutrients across the blood brain barrier.

Source: www.spirulina.com
Thus, *spirulina* with its whole spectrum of natural multi-nutritional properties can be used/explored as a therapeutic supplement in the management of various nutritional and metabolic disorders [Henrikson, 1997]. The various therapeutic effects of *spirulina* are discussed in table 6.

*Spirulina*, along with its wide spectrum of all the essential nutrients, also contains another nutrient - γ-linolenic acid (GLA), an essential fatty acid (in *spirulina*, of the 100% composition of essential fatty acid composition, about 24.9% is GLA). It is the precursor to the body's prostaglandins that are one of the most important hormones, which control many important functions in the body. Deficiency of GLA can occur due to high intake of saturated fats, increased consumption of alcohol or a diet that is deficient in GLA. Numerous studies have shown that GLA deficiency may lead to many degenerative diseases and health problems. In addition, its one such effect is on the inflammatory condition. Moreover, anti-oxidants (like tocopherol, β-carotene, superoxide dismutase) present in *spirulina* can quench the free radicals and stop oxidative damage also. Hence, its imperative that our diet contains GLA, anti-oxidants so that these properties of *spirulina* can slow/stop these health problems.

**Gamma linolenic acid (GLA)**

The essential fatty acids (EFA) act as the precursors of regulation of inflammatory function. These regulators are prostaglandins (PG) and leukotrienes (LT). They carry out many vital functions in the body, including the inflammatory and immune processes. Among the mediators generated by inflammatory cells and injured tissues, certain derivatives of phospholipids and fatty acids are important. Depending on the specific inflammatory cell and the nature of the stimulus, activated cells generate arachidonic acid (AA) by one of the two pathways (Figure 11) and when the synthesis of PG₂ and thromboxane X₂ (TX₂) series occur, they compete with the synthesis of LT₄ and LX₄ for the arachidonate substrate (i.e. phosphatidylcholine and phosphatidylinositol).
THERAPEUTIC EFFECTS OF SPIRULINA.

- Reduction of nephrotoxicity from drugs and heavy metals [Fukino, 1990; Yamane, 1988]
- Immune system stimulation from phycocyanin extracts [Schwartz et al., 1988; Babu, 1995; Baoyiang, 1994]
- Increased healthy lactobacillus flora
- Positive effect on Diabetes Mellitus [Mani et al, 2000; Parikh et al, 2001]
- Positive effect on Asthma [Labhe et al, 2001; Labhe et al, 2000]
- Benefits from high iron bioavailability in spirulina [Mani et al, 2000]
- Malnutrition recovery [Buaille et al 1990; Youghuanf, 1994]
- Anti-cancer and anti-tumour effect from β-carotene extract [Schwartz et al., 1988]
- Radiation protective effect from extract of spirulina [Qishen and Kolman, 1989]
- Effective against AIDS virus from sulfolipid extract of blue green algae [Gustafson, 1989]
FIGURE 11
METABOLISM OF ESSENTIAL FATTY ACIDS

Membrane Phospholipid

Stimulants:
- Angiotensin II
- Bradykinin
- Epinephrin
- Thrombin

Phospholipase A2

Arachidonic acid

Cyclooxygenase Pathway

Prostaglandins
- PGI₂
- PGF₂α
- PGE₂

Thromboxanes
- TXA₂

Lipoxygenase Pathway

5-HPETE
- LTA₄
- LTB₄
- LTC₄
- LTD₄
- LTE₄

Source: Modified from Rubin and Farber, 1988
The first pathway involves the stimulus-induced activation of phospholipase A2, which enhances the hydrolysis of AA from glycerol backbone of membrane phospholipids. In particular, phosphatidylcholine is an important substrate of phospholipase A2 and is thus the major source of AA in the inflammatory cells. The second mechanism by which AA is generated is through the metabolism of phosphatidylinositol by phospholipase C to diacylglycerol and inositol phosphates. Diacylglycerol lipase then cleaves AA from diacylglycerol. Once generated, AA, a polyunsaturated (20:4) fatty acid, is metabolised two pathways: Cyclooxygenation (with the help of cyclooxygenase enzyme), with the subsequent production of PG and TX; and lipoxygenation (with the help of lipoxygenase enzyme), to form monohydroxyeicosatetraenoic acids (HETE) and leukotrienes (LT).

Specific cyclooxygenase enzymes in the inflammatory cells generate endoperoxide derivatives of AA, including prostaglandin G2 (PGG2). This endoperoxide is unstable and, depending on the specific inflammatory cell or tissue, are metabolised to more stable prostaglandins, including PGI2, PGF2α, PGE2, PGD2, and TXA2. The primary cyclooxygenase metabolite in the platelets is TXA2, while endothelial cells secrete principally PGI2. PGI2 and PGE2, owing to their vasodilatory effects, enhance the vascular permeability at the sites of inflammation; TXA2 is a potent vasoconstrictor and plays an important role in the mediation of the 'second wave' of platelet aggregation.

A second pathway by which AA is metabolised in inflammatory cells and tissues is lipoxygenation and the formation of hydroperoxyeicosatetraenoic acid compounds (HPETE). HPETE compounds may be metabolised to hydroxyeicosatetraenoic acids (HETE) or to LTA4. The generation of LTB4 at the sites of tissue injury plays an important role in the recruitment of polymorphonuclear leukocytes. At the sites of tissue injury, production of LTC4, LTD4 and LTE4 also takes place. However, the importance of AA metabolites in mediating many of the effects of the inflammatory response is demonstrated by the ability of the inhibitors of the involved enzymes to
attenuate both the pathologic changes and clinical symptomatology. In this context, corticosteroids are widely used to inhibit the tissue destruction associated with many inflammatory diseases, rheumatoid arthritis and various types of glomerular nephritis. Corticosteroids induce the synthesis of an inhibitor of phospholipase A2 and block the release of AA in the inflammatory cells. A second class of anti-inflammatory agents that is widely used in the treatment of the inflammatory diseases comprises the non-steroidal anti-inflammatory drugs. These compounds include aspirin, ibuprofen that inhibit the cyclooxygenase, and thus the synthesis of PG and TX. (Here the role of spirulina can come in for attenuating the inflammation and can help in reducing the amount of drug consumed by nephrotic patients and also in decreasing the side effects of steroids).

Enrichment of diets with AA leads to the formation of PG and LT of 2 and 4 series that are pro-inflammatory in nature and have inflammatory effects in the body. The production of these pro-inflammatory series can be slowed/stopped, by incorporating essential fatty acid in the diet (as supplements) that will modify the production of various PG and LT (i.e. prostanoids of 1 and 3 series that have anti-inflammatory property). Hence, GLA, with its anti-inflammatory property plays an important rôle. Dietary GLA in the human body is produced through desaturation of linoleic acid by the action of the rate limiting enzyme linoleyl CoA desaturase. GLA further gets converted to dihomo-y-linolenic acid (DGLA), which further helps in the synthesis of PG of 1 series (Figure 12). Even though, the activity of linoleoyl-CoA desaturase is under hormonal and metabolic regulation but it has been shown that dietary fatty acids, alcohol and many other factors inhibit this enzyme, resulting in the deficiency of GLA, which turn leads to the impairment in the formation of PGEi. Hence, even if, the diet is rich in linolenic acid (LA) but the conversion of LA to GLA is poor and limited in the human body. And decreased concentrations of GLA have been reported in the patients with inflammatory
FIGURE 12
CONVERSION OF DIETARY GLA WITH THE HELP OF SPIRULINA INTO ANTI-INFLAMMATORY PROSTANOIDS

Dietary Gamma-Linolenic acid (GLA)

Di-homo Gamma Linolenic acid (DGLA)

Prostanoids of 1 and 3-series

Arachidonic acid

Cyclooxygenase

Lipoxygenase

PG &TXA₂

LT of 4-series

ANTI-INFLAMMATORY

PRO-INFLAMMATORY
diseases. Therefore, ingestion of a dietary supplement rich in GLA may help in increasing the concentration of DGLA, that may lead to increase in the production of PG of 1 series, which have anti-inflammatory properties (Johnson, 1997). GLA lowers the production of inflammatory eicosanoids by competing with AA as a substrate for cyclooxygenase activity and by inhibiting the conversion of linoleic acid to AA (Schwartz, 2000). Another beneficial effect of diet rich in GLA, that is metabolised to DGLA, is the inhibitory effect of GLA on LT synthesis. Therefore, diet rich in GLA may suppress the inflammation through the metabolism of GLA to DGLA and thus competitive inhibition of PGs of 2-series and LT of 4-series that are pro-inflammatory.

Many studies prove the diets supplemented with therapeutic doses of EFA have been found to be efficient in treating various inflammatory disorders. Fletcher and Ziboh (1990) reported that fish oil or vegetable oil rich in GLA could reduce the symptoms of inflammation in skin diseases. As discussed earlier, eicosapentaenoic acid (EPA) competes with AA for the metabolism by cyclooxygenase and lipoxygenase pathways. The selective metabolites derived from EPA have reduced the biological activities as compared to the AA-derived counterparts. Dietary supplementation with EPA led to the incorporation of EPA into the membrane phospholipids, along with the inhibition of 5-lipoxygenase pathway activity and also the reduction of the elaboration of platelet-activating factor. The neutrophil chemotaxis cells are substantially attenuated. This, therefore, suggests that EPA has anti-inflammatory potential (Lee et al., 1991) and clinical trials in rheumatoid arthritis have shown beneficial effects. Recent studies demonstrate that dietary GLA increases the content of its elongase product, dihomo-γ-linolenic acid (DGLA), within the cell membranes without concomitant changes in the arachidonic acid. Subsequently, upon stimulation, DGLA can be converted to PGE_1 by the inflammatory cells. This is noteworthy, as this compound possesses both anti-inflammatory and anti-proliferative properties (that will be useful in preventing proliferation associated with kidney diseases) (Fan and Chapkin, 1998).
Hypocholesterolemic property of *spirulina*

It is a well-known fact that the consumption of cholesterol rich food increases the blood cholesterol concentrations (including the atherogenic cholesterol LDL-C). And cholesterol accumulation in the arteries obstructs the flow of blood leading to various types of heart diseases. Moreover, well-published clinical studies indicate that as the concentration of cholesterol declines, the risk of heart attack and stroke decreases.

Hence, besides dietary improvements, search is under way for the identification of a safe and natural agent, which not only contains less concentration of cholesterol but also has the property to reduce the elevated cholesterol levels in the blood. Therefore, in this context, *spirulina* is an ideal choice, as it contains good amounts of GLA [www.spirulina.com]. The cholesterol reducing property of *spirulina* may be due its GLA content. Many well-published investigations have shown that dietary fish oil or fish consumption reduces the concentration of cholesterol (leading the health authorities to recommend more fish oil in the diet). The source of this beneficial EFA i.e. GLA in the fish and fish oil comes from the micro algae like *spirulina*, which is eaten by the fish. Furthermore, *spirulina* itself contains very less amount of cholesterol (10g of *spirulina* contains 1.3mg of cholesterol or 1g=0.13mg of cholesterol). Figure 13 depicts that *spirulina* has hypocholesterolemic property and can help in increasing the anti-oxidants status. All this in turn help in reducing the lipid peroxidation and lead to the reduction in the glomerular damage. Clinical investigations have proved that *spirulina* can effectively reduce the elevated blood levels of cholesterol [Kato et al., 1984]. Thus, *spirulina* with its whole spectrum of natural multi-nutritional properties can be used/explored as a therapeutic supplement in the management of various nutritional and metabolic disorders [Henrikson, 1997]. In our department numerous studies/clinical trial on the supplementation of *spirulina* in the management of various disorders have been carried
FIGURE 13
PREVENTION OF GLOMERULAR INJURY THROUGH SPIRULINA

† Oxidative Stress

SPIRULINA

Reduces cholesterol concentration

↓ LDL Cholesterol

Supplies dietary anti-oxidants

↑ Anti-Oxidant Status

↓ Lipid Peroxidation

↓ Glomerular Cell Injury
out. Mani et al (2000) carried out a study on the long-term effect of *spirulina* (2g/day) on the serum and lipid profile and glycated proteins in NIDDM patients. They observed a significant decrease in the levels of TG (p<0.01), TC (p<0.01), LDL-C (p<0.01) and VLDL-C (p<0.01) after two months of *spirulina* therapy. The control of glycaemia and lipidaemia in Type 2 diabetic patients through *spirulina* supplementation (2g/day for two months) revealed an appreciable reduction in the levels of TG (21.3mg%), TC (6.4mg%) and LDL-C (7.1mg%) with a marginal increase in the HDL-C (1.4%) level, was noticed in the study group. This resulted in a significant reduction in the atherogenic indices i.e. TC: HDL-C (p<0.05) and LDL-C:HDL-C (p<0.01) of these patients. Reduction in the concentration of apo B (16.1mg%, p<0.01), coupled with a rise in the concentration of apo A1 (11.4mg%, p<0.05) was seen in these patients. In addition, a significant increase in the ratio of apo A1:B was also observed in these patients [Parikh et al, 2001]. Favourable response after *spirulina* therapy was witnesses in the lipid levels of NIDDM patients after two months. The GLA content of *spirulina* may contribute to its hypocholesterolemic effect. It's an essential fatty acid, which may play a role in the prevention of fats and cholesterol accumulation in the body, thereby reducing the serum cholesterol concentrations. The decrease in the levels of TG and VLDL-C after *spirulina* supplementation could be attributed to decreased VLDL triglyceride production and increased clearance of VLDL in the periphery brought about by the high protein and fibre content of the *spirulina*. In diabetics, decrease in the fasting blood glucose levels may be due to rise in the insulin secretion because there is a definite stimulating effect of insulin on the lipoprotein lipase activity [Shils and Young, 1988]. The decrease observed in the VLDL-C concentration after *spirulina* supplementation would have resulted in lowering the LDL-C concentration, due to the fact that most of the LDL-C is formed from VLDL-C. A resultant significant lowering in the atherogenic indices of TC: HDL-C and LDL-C: HDL-C was consequently noted. These kinds of beneficial alterations in the lipid levels have been associated with a lower incidence of coronary heart disease (CHD). Apo A1 and B are the major protein components of HDL-C and LDL-
respectively. They have been the most frequently investigated as risk factor for CHD and spirulina supplementation helped in decreasing the apo B levels. Apart from all this, the beneficial role of antioxidants and superoxide dismutase can’t be ruled out.

Various other studies were carried out in the department to ascertain the impact of spirulina supplementation on the glycaemic and lipaemic responses of foods. In the various commonly consumed foods and snacks, rice-based recipes, cereal-based recipes and regional meals of India, spirulina was incorporated at a level of 2.5g. Its supplementation significantly decreased the two-hour post prandial glycaemic and lipaemic responses in the healthy and diabetic patients. From the above results it can be assumed that addition of 2.5g of spirulina in the above mentioned recipes helped in bring the insulin peak earlier. In addition, spirulina may posses some factor/factors that affects the digestion of carbohydrates, thereby, causing hypoglycaemic and hypolipidaemic effect. Therefore, the incorporation of spirulina in the diabetic diet would prove beneficial [Iyer et al, 1999; Mani et al, 1990; Mani et al, 1997]. Apart from this, beneficial role of spirulina therapy was also noticed in anaemic girls where the blood haemoglobin level was found to increasing [Mani et al, 2000]. Hence, spirulina with its possible beneficial effects has been shown to be effective in the management of various diseases. In Japan, clinical study on the anti-cholesterolaemic property of spirulina was conducted (Nayaka et al., 1986). For the study thirty male volunteers with high cholesterol, mild hypertension and hyperlipidaemia, showed effective lowering of serum cholesterol, triglycerides and LDL levels after the consumption of spirulina (4.2gm/day) for eight weeks. No changes in their dietary habits were made, except for the inclusion of spirulina to the diet. It was noticed that the level of serum total cholesterol significantly dropped from 244mg/dl to 233mg/dl.

Apart from all this, the beneficial role of antioxidants present in spirulina can’t be ruled out (Figure 13). Spirulina contains natural antioxidants
such as β-carotene, vitamin E and selenium and also superoxide dismutase (it is basically a metallo protein that catalyses the dismutation of superoxide free radical to molecular oxygen and hydrogen peroxide). By scavenging the free radicals in human beings, it offers protection from tissue damage [Venkataraman, 1992; Manoj et al, 1992] that effectively quenches the hazardous free radicals. These naturally occurring antioxidants have been extensively investigated for their antioxidant property [Miranda et al., 1998].

MANAGEMENT OF NEPHROTIC SYNDROME WITH MEDICATIONS

The incidence of nephrotic syndrome is approximately 2 per 100,000 children, with minimal change being the underlying histopathology in more than 85% of cases [Koskimies et al., 1982]. Overall maximum number of children respond to the initial steroid treatment [Koskimies et al., 1982; Tarshish et al., 1997] but there are few children who relapse back. Of the children who relapse, approximately half relapse frequently or become steroid dependent (definitions given in Appendix 2) [Koskimies et al., 1982; Tarshish et al., 1997]. These children are difficult to manage in clinical practice because of steroid toxicity.

For the management of paediatric nephrotic syndrome, the Indian Paediatric Nephrology Group and Indian Academy of Paediatrics have drawn consensus statement on the management of steroid sensitive nephrotic syndrome [Bagga, 2001]. The first line of therapy is the use of corticosteroids that is used for inducing remission in these patients. Prednisone and its active metabolite, prednisolone, have greater anti-inflammatory activity, cause less sodium retention than the natural hormone cortisol, and are the most widely used corticosteroid preparations in the current nephrology practice. The major therapeutic uses of prednisolone and prednisone are based on the anti-inflammatory and immunosuppressive activities of glucocorticoids. The suppression of inflammatory responses is independent of the initiating
stimulus and the action is mainly local. Some important components of the mechanism underlying the anti-inflammatory effects of corticosteroids are (i) inhibition of the adherence of neutrophils and monocytes-macrophages to the capillary endothelial cells of the inflamed area, (ii) blocking of the effect of macrophage migration inhibitory factor and (iii) inhibition of phospholipase A₂ activity thereby lowering the formation of prostaglandins, leukotrienes and related compounds.

Various treatment regimens have been used for the treatment of the initial episode of NS. But from the available literature [Hodson et al., 2000; ISKDC, 1981; Hogg et al., 2000; Bagga et al., 1999; Bargman, 1999; Cameron, 1998], the Expert Group recommends that the initial episode be treated with prednisolone administered in a dose of 2mg/kg in two-three divided doses daily for six weeks, followed by 1.5mg/kg as a single dose on alternate days for the next six weeks, after which the steroid is slowly tapered. When the nephrotic patient relapses, the patient should first be examined for infections, which are treated before initiating the corticosteroid therapy. The drug is administered in a dose of 2mg/kg/day (two divided doses) until urine protein is trace or nil for three consecutive days. Subsequently prednisolone is given in a dose of 1.5mg/kg/day on alternate days (QOD) for four to six weeks after which it is slowly tapered off. Nephrotic patients who are infrequent relapsers respond promptly to the treatment and are managed using aforementioned regimen for each relapse. Such patients are at a low risk for developing steroid toxicity. However, patients with frequent relapses or steroid dependence are managed in the above mentioned regimen for each and after remission the dose of prednisolone is tapered gradually to maintain the patient in remission on alternate day dose of 0.5mg/kg. In this case the patients have to be closely be monitored for growth, blood pressure and evaluation for features of steroid toxicity are essential. In general prolonged use of steroids have numerous side effects such as cushingoid faces, small stature, increased hunger, weight gain, mood changes, more chances of infections. Moreover, prolonged exposure to larger doses of prednisolone can
be diabetogenic with increased hepatic gluconeogenesis and glycogen storage. The peripheral utilisation of glucose is curtailed and there is resistance to insulin. As a result of anti-anabolic and catabolic action on proteins in peripheral tissues, there is increased availability of amino acids to the liver and a negative nitrogen balance. In addition, the glucocorticoids may also induce calcium loss by affecting the bone resorption and more importantly the renal excretory mechanism. In some children growth failure occurs that probably is a consequence of inhibition of the release of the growth hormone [Green et al., 1978].

If the prednisolone threshold, to maintain remission, is higher than 0.5mg/kg on alternate days, then the following immunomodulators are recommended.

(a) Levamisole
This may be administered in a dose of 2-2.5mg/kg on alternate day for 12-24 months along with it the treatment with prednisolone, 1.5mg/kg on QOD, is continued. Later on the dose of prednisolone is gradually reduced every four weeks to a maintenance does of 0.25mg/kg, which may be continued for six months. The chief side effects of treatment with levamisole is leukopenia and skin rash (may occur rarely). The total leukocyte count of the patient should be monitored every 4-8 weeks.

(b) Alkylating agents
Cyclophosphamide (2mg/kg/day) or chlorambucil (0.1-0.2mg/kg/day) are the alkylating agents that may be administered along with prednisolone (1-1.5mg/kg) on alternate days for twelve weeks. In view of larger experience and safer toxicity profile, cyclophosphamide is usually preferred. The total leukocyte count of the patient should be monitored every 2-3 weeks.

(c) Cyclosporin
This is administered in a dose of 4-5mg/kg daily for 12-24 months.
The therapy is continued with prednisolone in a dose of 1.5mg/kg QOD for 4 weeks and then tapered gradually. Side effects such as hypertension and hirsutism may occur.

SUPPORTIVE CARE / THERAPY

This forms an important aspect of managing the children with nephrotic syndrome. The management of nephrotic patients must take into consideration not only the specific pharmacological approach to the underlying glomerular disease, but also the supportive measures aimed at preventing and treating the clinical sequelae of massive proteinuria. This supportive care/therapy is of importance for those patients who do not respond to immunomodulating agents and are therefore, exposed to the complications that occur due to prolonged duration of nephrotic syndrome. This supportive care/therapy can be in the form of managing oedema, hypoalbuminaemia and hyperlipidaemia with the help of drugs and dietary management of these patients.

(a) Oedema

Control of oedema is an integral part of the supportive care. Treatment with corticosteroids usually leads to diuresis within 48-72 hours. Diuretics are thus avoided unless oedema is significant and should not be used in children with diarrhoea, vomiting of hypovolemic.

Many patients, however, particularly those with anasarca or volume overload do not respond to thiazides. Therefore, in such cases loop diuretics such as frusemide are needed/essential. Frusemide is administered in a dose of 1-3mg/kg/day. An additional treatment with potassium sparing diuretics (e.g., spironolactone) is not required if frusemide is used in this dose for less than one week. On the other hand, patients requiring higher doses and prolonged duration of treatment with frusemide should receive spironolactone (thiazide agent)(dose 2-4mg/kg/day). Blood pressure should be monitored
frequently. In the end, a gradual reduction of oedema, over one week, is preferred.

(b) Hypoalbuminaemia

The use of human serum albumin to correct hypoalbuminaemia is generally fruitless and expensive undertaking for the rates of urinary albumin loss are such that any advantage gained is transitory. Therefore, it is recommended that hypoalbuminaemia be managed through dietary supplements. Nevertheless, if the need arises then albumin infusions of 1-2g/kg are appropriate, usually given as a 20% solution.

(c) Hyperlipidaemia

One of the cardinal clinical features of nephrosis is the aberration witnessed in the lipid profile of these patients. It is well accepted that the cardiovascular risk factor present the same hazard to a patient with nephrotic syndrome as they do to a patient without renal disease. The decision to treat the patients must be based on the extrapolations from the clinical studies, taking into account the assessment of an individual patient's risk profile and prognosis - age, gender, cardiovascular history, family history and concomitant risk factors like hypertension.

Various lipid-lowering regimens have been tried in the patients with renal disease, which includes dietary regimen (low saturated fat diets, fish oil supplements and life style modifications) and medication regimen. Of all the hypolipaemic drugs available, Hydroxymethylglutaryl Co-enzyme A (HMG-CoA) reductase inhibitors have been most widely used class of drugs for the treatment of hyperlipidaemia complicating chronic renal disease [Matzkies, 1999; Oldricht et al., 1999]. The various HMG-CoA reductase inhibitors that have been investigated are Lovastatin, Simvastatin, Fluvastatin and Provastatin. These drugs are the most easily tolerated and the most efficacious agents for reducing the elevated LDL-C levels. The HMG-CoA reductase inhibitors act by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A,
the rate-limiting enzyme of cholesterol biosynthesis. This causes a sequence of steps having the end result of increasing the LDL receptor activity on hepatocytes and thereby speeding the clearance of LDL-C from the plasma. Although increased clearance of LDL-C by LDL receptors is the most important effect of the statins, they may also reduce the production and enhance the hepatic clearance of VLDL-C. This would account for the triglyceride reductions observed when these drugs are used. A crucial consequence of inhibition of biosynthesis of cholesterol within hepatocytes is a reduction in intracellular cholesterol stores. Homeostatic mechanisms within the hepatocytes then increase the LDL receptor activity on the cell membrane and LDL-C is cleared more rapidly from the circulation, bringing its cholesterol content into the hepatocyte. No serious side effects of these cholesterol-lowering drugs have been documented in any of the clinical trials. However, the side effects of these drugs may be aggravated in the presence of renal failure or any renal disease and in the patients with past history of liver disease. Periodic liver function tests have to be performed in the patients consuming these drugs.

**DIET AND NEPHROTIC SYNDROME**

Nephrotic syndrome occurs when the filters in the kidney leak an excessive amount of protein in urine. The level of protein in the blood then falls and this allows fluid to leak across very small blood vessels into the tissues. Swelling around the eyes, abdomen and legs is the consequence of this process. The disease is characterised by proteinuria (Excessive protein loss especially albumin in the urine), low blood protein and oedema (swelling in the tissues). Protein losses in the urine are commonly 5-10gms per day.

**NUTRITIONAL MANAGEMENT**

One of the aims of nutritional management is to replace as much of the protein loss in the urine by an intake of good quality protein (www.pediatriconcall.com). The dietary modification form one of the supportive care that is needed in the nephrotic patients and are as follows:
Protein

The recommended level of protein remains a matter of controversy. Historically, patients have received diets high in protein (up to 1.5 g/kg per day) in an attempt to increase serum albumin and to prevent malnutrition resulting from urinary protein losses. This strategy has not been effective in increasing serum protein levels, primarily because the resultant enhancement in proteinuria negates the beneficial effect of increased albumin synthesis. And it was observed that a very high protein diet might cause tubular damage to the kidneys, as the kidneys will have to filter more proteins [Palmer, 1993]. However, studies have shown that a reduction of protein to as low as 0.6 g/kg per day can decrease proteinuria without adversely affecting serum albumin. Of the protein, 75% should be of high biological value. In presence of malnutrition, good renal function, and massive proteinuria: allow intake \( \leq 1.5 \) of proteins g/kg. In some cases, a range of 0.6 to 1.0 g/kg of ideal body weight, depending on CCrCl and nutritional status, is recommended, plus gram-for-gram-replacement of urinary protein losses. In presence of reduced CCrCl, follow the guidelines for chronic renal failure, with additional grams of protein to match 24-hour urinary protein loss.

For children, the Dietary Reference Intake for age with the addition of urinary protein loss is recommended. Since Indian children usually have a low intake of protein (even less than the RDA), adequate protein intake — upto 2gm/kg/day in children and not more than 3gm/kg/day in infants is advocated.

Calories and fats / oils

Hyperlipidaemia is a common manifestation of the nephrotic syndrome. The mechanism probably involves increased hepatic synthesis in response to decreased serum proteins as well as defective peripheral utilization of fat. Since the hyperlipidaemia of nephrotic syndrome is a secondary manifestation, the impact of a fat-modified diet is probably insignificant. But a high calorie diet to conserve protein in the body should be necessary (so that proteins are not utilised for energy in the absence of fats).
However, fat content is not increased in the diet as patients with nephrotic syndrome as they have high serum triglycerides. In addition, it is recommended that as part of the initial general healthy eating advice, oils and saturated fats (ghee, butter, margarine, coconut oil) should be avoided (www.healthsystem.virginia.edu) combined with weight loss (in obese patients) is recommended since these patients have an increased risk of cardiovascular disease. Moreover, in the nephrotic child who has been on long-term corticosteroids, it may however, be necessary to reduce the energy intake with low carbohydrate diet to prevent the occurrence of obesity in these patients.

**Sodium**

The level of sodium prescribed is based on severity of oedema and hypertension. To prevent massive oedema, sodium levels in the diet must be low. Generally, sodium is restricted to 1 to 3g daily. But usually 500mg sodium diet is satisfactory (http://www.healthsystem.virginia.edu). Salt should be restricted but diets should be palatable. But from a practical point of view, it is generally sufficient to recommend not adding salt in the diet of these children [Ponticelli and Passerini, 1994].

**Vitamins and minerals**

Supplemental zinc may be indicated, as zinc deficiency is common in nephrotic patients. Supplemental vitamin D and iron (for women) may also be needed to normalise serum levels. Supplement 1 to 1.5 g of calcium. Also supplement B vitamins (niacin, riboflavin, and thiamine) (http://www.healthsystem.virginia.edu).

Thus, diet of nephrotic patients should have proteins that have high biological value, fewer amounts of saturated fats along with vitamins and minerals. When all these nutrients are provided to these patients through diet, then the clinical manifestations of nephrotic syndrome, which are hypoproteinaemia and hyperlipidaemia can be decreased. In addition, the use
of an anti-inflammatory agent may reduce the inflammation in the kidney and antioxidants may prevent the oxidation of LDL-C that may otherwise could have led to further kidney damage. Thus, in this context, the encapsulated miracle 'spirulina' with its array of all the essential nutrients can be an effective therapy for combating inflammation, hypoproteinaemia and hyperlipidaemia in nephrotic patients. Hence, the present study was planned with the broad objective of 'ascertaining the therapeutic effect of spirulina on the patients suffering from nephrotic syndrome'.
**KEY POINTS**

✧ The hallmark of nephrotic syndrome is proteinuria.

*Eddy et al, 1995* and *Kean and Eknoyan, 1999*: Proteinuria is not only a marker of glomerular damage but is toxic to the kidneys.

✧ Structural changes that occur within the GBM which lead to the development of proteinuria are

1. Effacement of the podocyte foot processes

*Kurihara et al, 1992*: Foot process effacement results in a decrease in the filtration slit frequency along the GBM and has been associated with narrowing of the filtration slit and development of tight junctions in between the foot process.

2. Depletion of polyanion

*Bridges et al, 1982*: They observed a 50% reduction of the fixed negative charge in MCNS patients.

*Vernier et al, 1983*: Noticed that depletion of polyanion was observed in congenital nephrotic syndrome.

✧ Prolonged proteinuria leads to the development of hypoproteinaemia. The plausible explanations are

1. Synthesis of albumin during nephrosis could be diminished or increased.

*Kaysen et al, 1986*: Albumin synthesis by isolated perfused livers is inversely proportional to the osmotic pressure of the perfusate. It is increased at low osmotic pressure and is decreased when the osmotic pressure is increased.

2. Changes in the catabolism of albumin

*Reed, 1981*: He observed that the absolute rate of albumin catabolism is reduced.
Park et al, 1984: The decrease in the rate of albumin catabolism is actually greater than the increase in the rate of albumin synthesis in these patients. Although, it is possible that the renal component of albumin catabolism is increased in nephrosis, and may lead to the absolute contribution of the kidney to albumin catabolism.

✧ When the level of albumin decreases (hypoalbuminaemia) in the plasma, the colloid osmotic pressure reduces and this turns causes the transudation of fluids from extra-cellular into intra-cellular compartments thus resulting in oedema.

✧ Hyperlipidaemia is commonly observed in these patients. Various theories have been postulated and are following

1. Enhanced hepatic synthesis
   Brenner and Shafrir, 1982; Gherardi et al, 1980; Marsh and Drabkin, 1960: Reported that the abnormal glomerular permeability causes increase in the permeability of plasma proteins and diminished plasma oncotic pressure, (all) may contribute to the enhanced hepatic synthesis of albumin along with the other lipoproteins i.e., cholesterol, triglyceride might be enhanced in parallel with the apolipoproteins.

Warwick et al., 1990; Warwick et al., 1991; Moorhead et al., 1989: Furthermore, by trace-labelling the apo B moiety of VLDL and LDL, it has been possible to demonstrate higher turnover and increased absolute catabolic rates of these lipoproteins, which implies that their rate of synthesis is increased.

2. Defective/impaired lipoprotein catabolism
   Measurements of LPL activity have demonstrated impaired enzyme function, and this has been presumed to be a major cause of the catabolic defect. Reduced activity of LCAT has also been documented in human
plasma. Gherardi et al, 1980: The activity of this enzyme may be depleted as a result of its loss in urine.

3. Defective receptor clearance of lipoprotein particles

A further abnormality that may contribute to nephrotic hyperlipidaemia is the defective removal of IDL-C and LDL-C from the circulation via lipoprotein receptors.

Warwick et al, 1990: Reported a 55% reduction in the receptor-mediated clearance of LDL-C and an increased catabolism via alternative pathways.

 Patients with increased duration of nephrotic syndrome may be at the risk of developing cardiovascular disease.

Wheeler and Bernard, 1994; Majumdar and Wheeler, 1994; Kean et al, 1991: In addition, recent experimental evidence supports the hypothesis that lipids contribute directly to glomerulosclerosis and tubulointerstitial injury and correction of lipid abnormalities will slow the progression of nephrosis to chronic renal failure.

 The main aim of therapy for these patients has moved from symptomatic relief towards long-term preventive treatment to help these patients to remain in remission for a longer time. As increasing side effects of the drugs on longer use have been observed and also to correct the lipid abnormalities, improve the protein status and halt the further progression of the disease, there is a need for alternative therapies that lack the adverse effects and help in bring down the elevated lipid levels and steadily improves the protein levels in these patients. In this context, the current study was carried out to ascertain the effect of spirulina in the management of nephrotic syndrome.
Spirulina, with its spectrum of all the essential nutrients can be used as a therapeutic supplement in the management of nephrotic syndrome.

Mani et al [1990-2000] have extensively conducted an array of studies that have shown the beneficial effects of spirulina in the treatment of hyperlipidaemia, diabetes, anaemia and asthma. Iwata, 1990; Kato et al, 1984; Devi and Venkataraman, 1983; Chen et al, 1981: Clinical investigations have shown that spirulina can effectively reduce the elevated blood levels of cholesterol.

Spirulina contains GLA and the cholesterol reducing property of spirulina can be attributed to its GLA content.