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Case Report

Membranoproliferative Glomerulonephritis in Systemic Sclerosis of Childhood

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Summary. The renal lesions of a 5-year-old girl with progressive systemic sclerosis are described. The nephropathy was clinically characterised by moderate proteinuria, microscopic hematuria and transient hypertension. Light microscopy showed membranoproliferative glomerulonephritis of segmental character. On electron microscopy intramesangial, subendothelial and extramembranous glomerular deposits were observed. By immunofluorescence microscopy deposits of IgG, Clq, C_4, C_3, C_5, C_8 und C_9 in a predominantly subendothelial location were found in all glomeruli. Vascular lesions were of minor degree. Histological and immunohistological findings are compatible with an immune complex disease.

Key words: Progressive systemic sclerosis in childhood – Segmental membranoproliferative glomerulonephritis – Immune complex disease.

Introduction

Progressive systemic sclerosis (PSS) or scleroderma is a connective tissue disease affecting the skin, synovial membranes and the blood vessels of various visceral organs. Females are predominantly affected, with rates three times higher than males (Tuffanelli and Winkelmann, 1961; Medsger and Masi, 1971). In childhood only a few cases of PSS have been reported in the literature (Tuffanelli and Winkelmann, 1961; Jaffe and Winkelmann, 1961; Kennedy, 1964; Mukherjee et al., 1966; Kass et al., 1966; Beetham et al. 1967; Barnett et al., 1969; Medsger and Masi, 1971; Velayos and Cohen 1972; Ansell et al., 1976; Stögmann et al., 1976). Localized cutaneous forms of sclerosis are observed much more frequently than PSS in the pediatric age group (Jaffe and Winkelmann, 1961; Chazen et al., 1962; Kass et al., 1966; Hanson et al., 1974; Ansell et al., 1976; Stögmann et al., 1977).

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Renal involvement is an inconstant finding in children with PSS and usually manifests itself at an advanced stage of the disease by proteinuria, hematuria, hypertension and renal failure (Kass et al., 1966). In two children with PSS nephropathy in whom kidney specimens were examined, the histopathological lesions were characterized by mucoid intimal thickening of the interlobular arteries, fibrinoid necrosis of arterioles and glomerular capillary loops and macroscopically by focal ischemic necrosis of the kidney cortex (Kass et al., 1966).

These lesions are identical to those described in adult patients with PSS (Heptinstall, 1974). In one child without clinical manifestations of renal involvement the kidney histology was normal (Mukherjee et al., 1966).

The observation of PSS in a 5-year old girl with cardiovascular and renal involvement gave us an opportunity to analyse the kidney lesions shortly after the onset of the disease. In contrast to previous reports the vascular lesions were found to be of minor degree only and the glomerular changes seem to indicate an immune complex disease.

Clinical History

At the age of 4 years and 5 months diminished mobility of the lower extremities were noted in a girl. Two months later an attack of abdominal pain occured with fever up to 39° C and pains in the thighs, without any signs of infection. Because of cardiac failure with peripheral and pulmonary edema and urinary urgency the child was admitted to the University Children's Hospital in Ulm (Prof. Dr. W. Teller). The diagnosis of PSS was made. After vigorous treatment cardiac failure improved. Proteinuria (up to 1.1 g/24 h), hematuria (up to 1200 erythrocytes/ml) and hypertension (up to 160/100 mm Hg) were noted and the girl was referred to our unit for renal biopsy.

On admission at the age of 4 years and 9 months the child appeared in a fair general condition and nutritional state. The mental and somatic development were normal (body weight 17.0 kg, height 104 cm). The skin was partly tense and atrophic. Active and passive movements in almost all joints of the upper and lower extremities, especially the wrists, elbows and hips, were greatly diminished and painful. The hands were more or less fixed in a claw-like position and the child was unable to walk without support. Histology of the skin was compatible with early changes of scleroderma. Splitlamp examination was normal. Electrocardiogram showed depression of T-waves and the measurement of systolic time interval a pre-ejection phase twice the normal value (Dr. H.E. Ulmer).

Laboratory Findings

Blood Counts. Hb 10.7 g%, erythrocytes 4.0 millions/mm^3, hematocrit 30%, leucocytes 8.400/mm^3 with eosinophilia up to 18%, sedimentation rate 44/84 mm.

Serum Chemistry. Total proteins 6.35 g%, albumin 3.8 g%, IgA 1.9 mg/ml, IgG 24.5 mg/ml, IgM 2.32 mg/ml. Urea-N 10.8 mg%, creatinine 0.6 mg%, normal electrolytes, SGGT up to 24 µ/l, SGOT up to 81 µ/l, SGPT up to 65 µ/l, aldolase 75 µ/l, CPK 7 µ/l, LDH 291 µ/l, HBDH 260 µ/l. Normal renin activity and aldosterone values in the peripheral blood, normal aldosterone excretion rate.

Coagulation Studies. Thrombocytes 246,000/mm^3, partial thromboplastin time 47 s, thrombin time 25 s.

Urine Analysis. Proteinuria up to 0.7 g/24 h, clearance IgG/Transferrin 0.15 (selective), constant microscopic hematuria, aminoacid excretion normal.

Renal Function Tests. Inulin clearance 63 ml/min/1.73 m^2, PAH-clearance 381 ml/min/1.73 m^2, maximal osmolality after 12 h thirst 600 mosm/l.

Immunological and Complement Findings

Autoantibodies against smooth muscle cells positive at a titer 1:40. The following tests were negative or normal: Antinuclear factors, DNA-antibodies, rheumatoid factor, C-reactive protein, anti-glomerular basement membrane antibodies, Australia antigen, indirect Coombs test. Normal values for CH 50, C4 and C3. C3Nef was negative, stimulation by mitogens and mixed lymphocyte culture failed to show any indication for a defect in T-cell function.

Clinical Course

Drug therapy by digitalis and diuretics was followed by rapid improvement of the cardiovascular signs. Blood pressure returned to normal values. After physiotherapy and prednisolone (0.3 mg/kg every second day) the arthritic signs and skin changes improved. Slight proteinuria, microscopic hematuria and reduction of GFR persist to date now 5 years 6 months.

Material and Methods

For light microscopic examination renal tissue was fixed for 6 h in Dubosq-Brazil fluid and subsequently for 24 h in 12% formalin. Paraffin sections were stained with hematoxylin and eosin, trichrome light green, periodic-acid-Schiff with hematoxylin and chromotrope silver methenamine. For electron microscopy the kidney specimens were fixed in collidin buffered 1% OsO_4 and phosphate buffered 2.5% glutardialdehyde, postfixed in OsO_4 and embedded in araldite. Semithin sections were stained with toluidine blue and paraphenylendiamine, ultrathin sections with lead citrate and uranylacetate (Reynolds, 1963) as well as with silver methenamine.

For immunohistological examination kidney and skeletal muscle tissue was snap frozen in isopentan precooled in liquid nitrogen. 5 µm cryostat sections were incubated with the following antisera: Anti-human-IgG, -IgA, -IgM, -IgD, -IgE, -albumin, -fibrin, -C4 and -C3, FITC labelled (Behring-Werke, Marburg); from commercial antisera against C1q, C5, C9, factor B, factor VIII associated antigen (Behring-Werke) and C8 (Cordis) the IgG fraction was isolated and labelled with FITC (Sylvana). Anti-properdin was kindly provided by Dr. Jungfer, Heidelberg.

Results

I. Kidney Biopsy

Light Microscopy. 18 glomeruli were avaible. Most of them were enlarged and showed a focally accentuated lobular pattern. One glomerulus was completely sclerosed. Mesangial cell proliferation was prominent, but irregularly distributed from one intercapillary, area to another and was associated with a marked increase of mesangial matrix. Endothelial cells exhibited varying degrees of cytoplasmic swelling. Occasionally a polymorphonuclear leucocyte was found in the capillary lumen. Some podocytes were hypertrophied but no crescent formation nor segmental necrosis of the glomerular tuft could be observed (Fig. 1a, b).

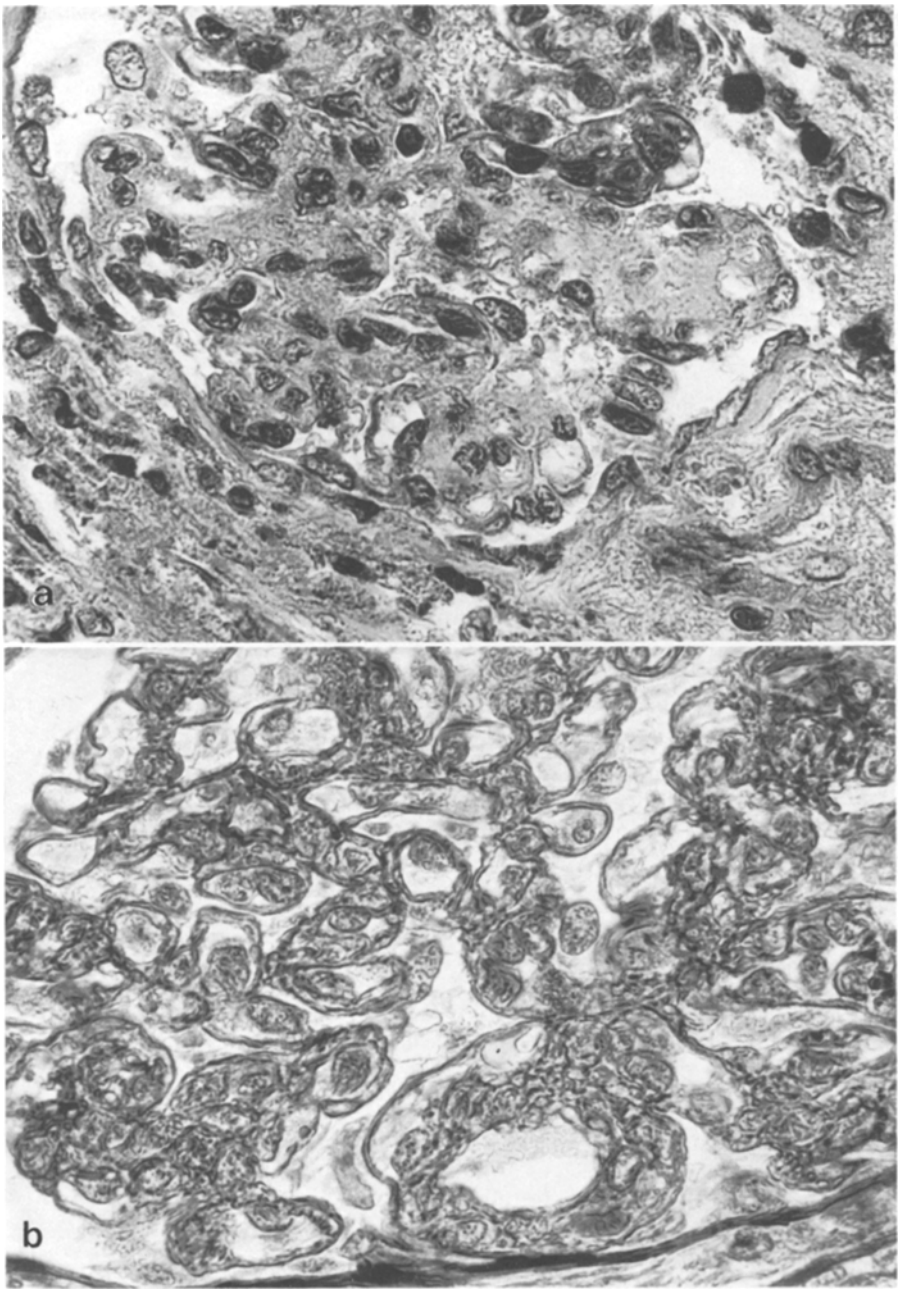

Fig. 1. a Moderate mesangial hypercellularity. Increase in mesangial matrix. Segmental thickening of glomerular capillary walls. Trichrome stain. ×800. **b** Segmental reduplication of glomerular basement membranes on silver impregnation. ×800

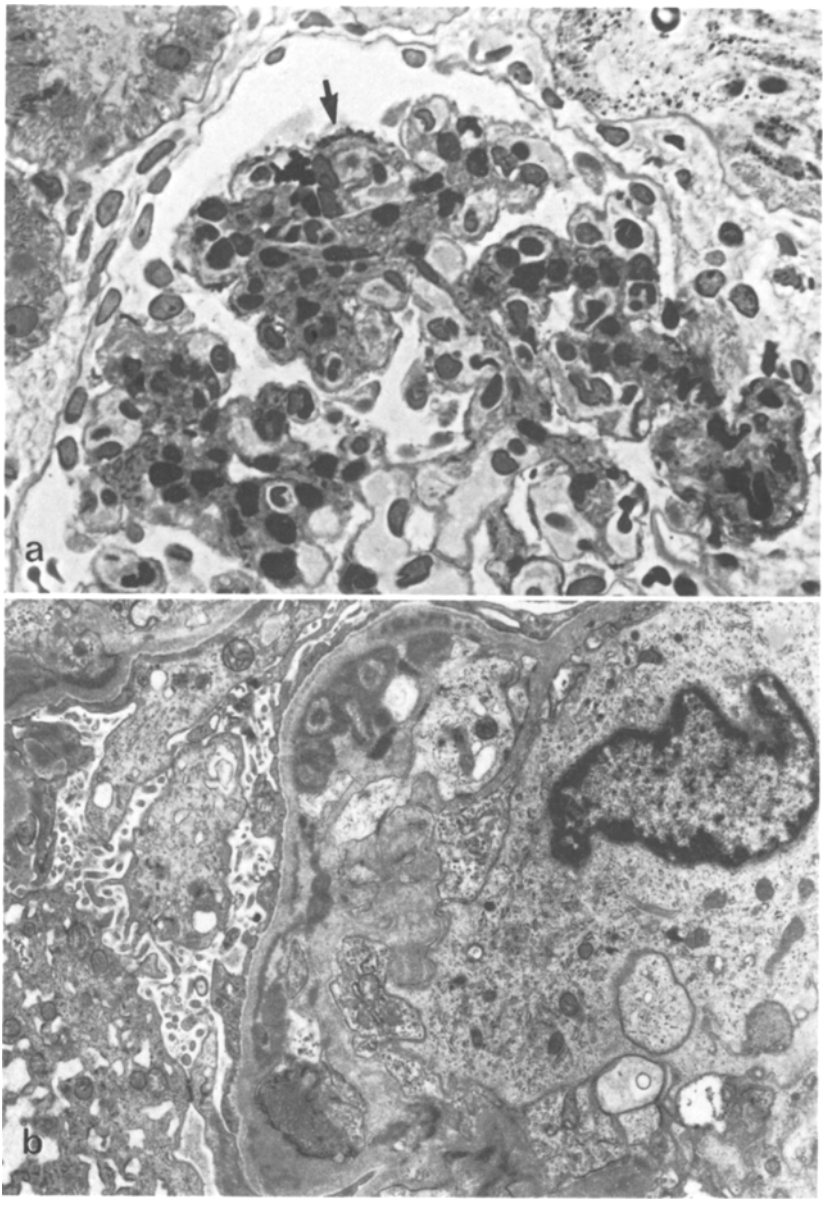

Fig. 2. a Irregular mesangial enlargement with proliferation of mesangial cells. Segmental basement membrane thickening, occasionally with epimembranous deposits (↑). Semithin section, toluidine blue and paraphenylendiamine. ×600. **b** Subendothelial and transmembranous osmiophilic deposits. Circumferential mesangial interposition with a newly formed basement membrane layer. Electron micrograph. ×6800

Many of the glomerular basement membranes showed an irregular thickening and splitting. On silver impregnation these capillary walls appeared with double contours forming a translucent space between original membranes and reduplicated membrane layers. On semithin sections subendothelial and occasionally extramembranous deposits could be demonstrated. Other basement membranes were thin and showed no abnormal deposition (Fig. 2a).

A few proximal convoluted tubules were markedly dilated and contained hyaline and hemoglobin casts. A small focus of tubular atrophy and interstitial fibrosis was noted in the subcortical area. Vascular lesions were non-characteristic. Some interlobular arteries and more often afferent arterioles showed hypertrophy and swelling of their endothelial cells. Minimal hyperplasia of media smooth muscle cells was the only finding in larger arteries.

Electron Microscopy. In all glomeruli an increase of mesangial ground substance and irregular proliferation of mesangial cells narrowing the glomerular capillary lumen were found. Endothelial cells exhibited varying degrees of cytoplasmic swelling and a decrease in their number of cell organelles. Segmental fusion of epithelial foot processes was present along the glomerular basement membranes. Irregularly distributed electron dense deposits were observed in all glomeruli. They were situated in the mesangial matrix beneath the surrounding basement membranes and in many segments along the capillary walls in subendothelial, occasionally trans- and epimembranous locations. Spike formations were missing. In some glomerular capillary loops mesangial basement membrane substance extended along the peripheral membranes forming a new membrane layer resulting in a reduplication and double contour appearance of the capillary walls (Fig. 2b). Proximal tubular epithelial cells frequently contained hyaline droplets. The only vascular finding consisted of a marked swelling and hypertrophy of endothelial cells of afferent arterioles and interlobular arteries. No deposits could be demonstrated in the arterial walls.

Immunofluorescence Microscopy. Fourteen glomeruli were examined. In all of them subendothelial deposits of IgG, C1q, C3, C4, C5, C8 and C9 were present. The deposits varied in size and fluorescence intensity. They were continuous along the capillary walls or coarsely granular in distinct locations. No deposition of properdin, factor B or fibrin was found. A few scattered deposits of IgM and IgA were also observed in subendothelial distribution (Fig. 3a–d). Blood vessels did not fix either immunoglobulin nor fibrin antisera. Positive fluorescence was only found for C9. Fixation was pronounced at the glomerular vascular pole and was present in minor degree in the walls of interstitial capillaries. C9 deposits in a granular or confluent pattern could also be demonstrated on some tubular basement membranes.

II. Skeletal Muscle Biopsy

By *light microscopy* focal mononuclear infiltration and minimal proliferation of fibroplasts could be demonstrated in the interstitium, but no degenerative changes nor atrophy of the muscle fibers were found.

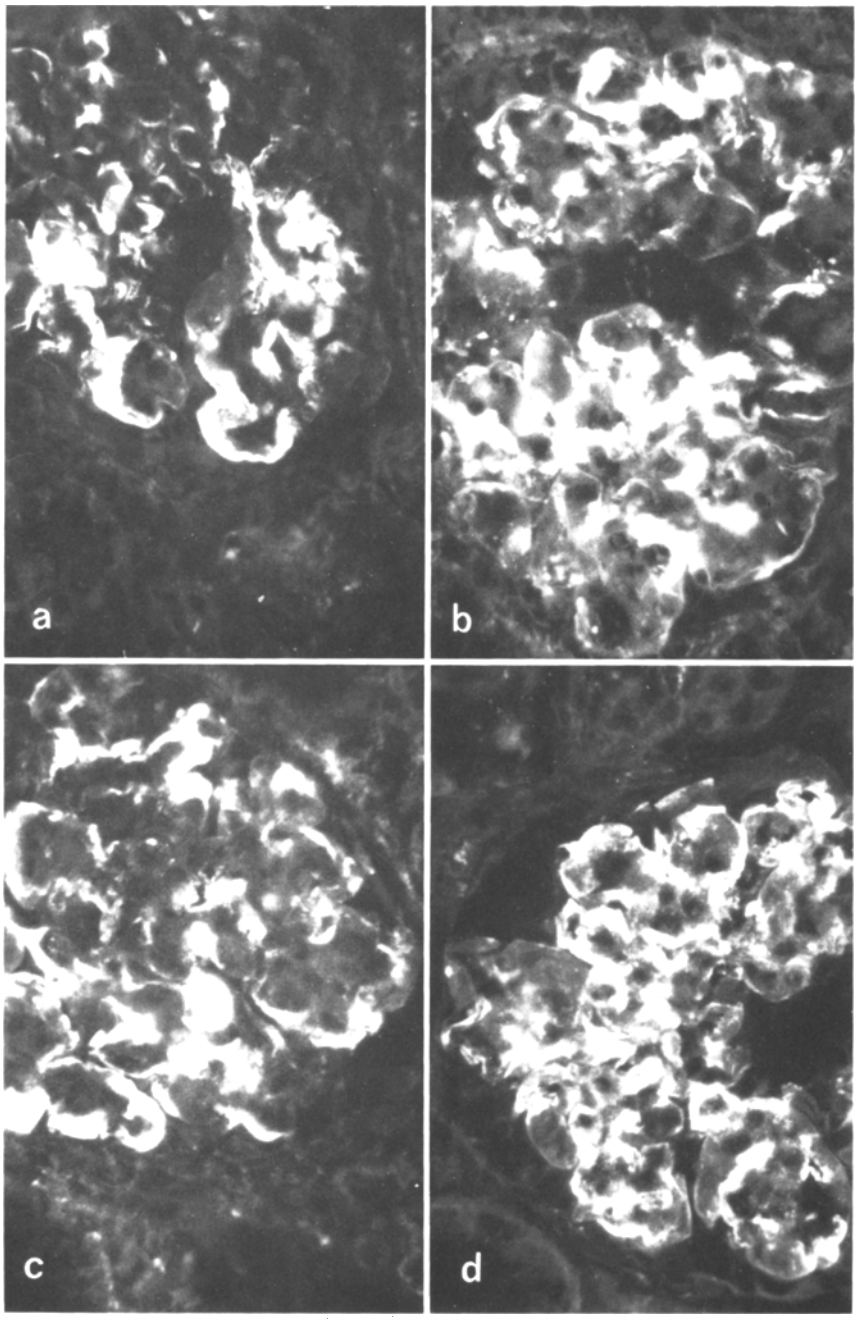

Fig. 3a–d. Immunofluorescence microscopy. Predominantly subendothelial deposition of IgG (**a**), C3 (**b**), C1q (**c**) and C9 (**d**). ×430

Immunofluorescence studies for immunoglobulins and complement were negative. Only perivascular deposits of fibrin and increased fluorescence with antiserum to factor VIII associated antigen could be demonstrated in vascular endothelial cells.

Discussion

In childhood scleroderma is a rarity. Of the 727 cases reported by Tuffanelli and Winkelman (1961) only 1.5% of the patients had onset during the first decade and 7.3% during the second decade of life. Barnett and Coventry (1969) found two of 60 patients before the age of twenty and in the report of Medsger and Masi (1971) only one female patient developed PSS before the age of 15 years.

In most studies on children with PSS renal involvement was not observed (Jaffe and Winkelman, 1961; Kennedy, 1964; Mukherjee et al., 1966; Beetham et al., 1967; Velayos and Cohen, 1972; Ansell et al., 1976; Stögmann et al., 1977). Only Kass et al. (1966) in a large survey of 16 children with PSS presented four patients with renal disease. The incidence of renal involvement in adults with PSS varies from 5% to 60% of the patients depending on the clinical definition of nephropathy (Tuffanelli and Winkelman, 1961; Medsger and Masi, 1973; Cannon et al., 1974; Oliver and Cannon, 1977). In the presence of renal symptoms prognosis is poorer and in fact, most deaths in patients with PSS are due to renal failure, in children as well as in adults (Kass et al., 1966; Medsger and Masi, 1973; Cannon et al., 1974; Heptinstall, 1974; Oliver and Cannon, 1977). In the end-stage, regular dialysis and renal transplantation have been performed successfully in adults (Richardson, 1973; Cannon et al., 1974; Merino et al., 1977) but in some cases PSS lesions recurred in the transplanted kidney (Woodhall et al., 1976; Merino et al., 1977).

The renal pathology described in PSS is rather uniform (D'Angelo et al., 1969; Vidt et al., 1971; McGiven et al., 1971; Cannon et al., 1974; Heptinstall, 1974; Kincaid-Smith, 1975; Oliver and Cannon, 1977; Ehrenfeld et al., 1977). The most characteristic findings are observed in the interlobular arteries and afferent arterioles: mucoid intimal proliferation with concentric hyperplasia of intimal cells embedded in large amounts of mucoid or myxomatous ground substance and concentric argyrophilic fibrils, leading to narrowing or obliteration of the vascular lumen. The ground substance has staining characteristics of glycoprotein and mucopolysaccharide (Fisher and Rodnan, 1958). Fibrinoid necrosis, medial smooth muscle hyalinisation and intimal cell proliferation develop in the afferent arterioles. The vascular changes cannot be distinguished from those observed in malignant nephrosclerosis (Fahr, 1919; Fisher and Rodnan, 1958; Ehrenfeld et al., 1977). The pathogenesis of the renal vascular lesions in PSS is unknown; they are found in the absence of elevated blood pressure (D'Angelo et al., 1969; Vidt et al., 1971; Kincaid-Smith, 1975) so that an important role for reduction in renal blood flow in the development of the changes has been emphasized (Cannon et al., 1974; Oliver and Cannon, 1977). Glomeruli may be also affected in PSS, with different degrees of ischemia or even necrosis

of the glomerular tufts. In the acute stage subendothelial fibrin deposits have been reported (Kincaid-Smith, 1975), followed by reduplication of the capillary basement membranes and collagen fibers within the capillary walls in the healing stages. Furthermore, a segmental wire-loop appearance of glomerular capillary walls was observed in some patients (D'Angelo et al., 1969; Vidt et al., 1971; McGiven et al., 1971).

The histological picture in the kidney of our patient differs from that usually found in PSS. Vascular lesions are limited to swelling and minimal hyperplasia of intimal cells in interlobular arteries and afferent arterioles. In contrast, glomerular involvement is prominent and corresponds to segmental membranoproliferative glomerulonephritis with predominantly subendothelial deposits.

Immunofluorescence microscopic findings of PSS kidney have been mentioned only rarely in previous reports. In the arterial and arteriolar walls isolated deposits of fibrin (Fenell et al., 1961; Ehrenfeld et al., 1977) or immunoglobulins (Freedman et al., 1960), usually associated with complement components (McGiven et al., 1971; Gerber, 1975; McCoy, 1975; Woodhall et al., 1976) were detected. Likewise immunoglobulins – predominantly of the IgM class – and complement were demonstrated in the glomeruli of some patients with PSS (Freedman et al., 1960; Vidt et al., 1971; McCoy et al., 1975; Woodhall et al., 1976; Merino et al., 1977). The immunohistological picture of our case with predominantly subendothelial deposits of immunoglobulins, mainly IgG, and complement components C1q, C4, C3, C5, C8, C9 differs from earlier findings and suggests that the glomerular lesions are caused by immune complex deposition in the kidney.

The etiology of PSS is unknown. There is a clinical overlap with other collagen diseases such as lupus erythematosus, dermatomyositis or rheumatoid arthritis (Sönnichsen et al., 1977). Antinuclear factors, DNA antibodies (Jablonska and Cherzelski, 1972; Meffert et al., 1975; Ansell et al., 1976; Sönnichsen et al., 1977; Stögmann et al., 1977) and positive rheumatoid factor (McGiven et al., 1971; Hanson et al., 1974) are frequent imunological findings in patients with PSS. Antinuclear antibodies were also demonstrated in the eluate from kidney specimens (McGiven et al., 1971; Woodhall et al., 1976). A disturbance of cellular immunity with a decrease of T-lymphocytes and a relative increase of B-cells has been reported in two children with PSS by Stögmann et al. (1977).

In our case the detection of low titers of smooth muscle antibodies was the only positive serological finding suggesting an immunological background. The presence of these antibodies, however, is uncharacteristic and may be observed in other collagen diseases and during non-specific infections. Its relationship to the renal lesions is thus doubtful. A hypocomplementemic membranoproliferative glomerulonephritis is excluded by the presence of normal serum complement levels and by the absence of C3-nephritis factor. There was no indication of systemic lupus erythematosus where a similar morphological pattern might be expected. In view of these negative findings the pathogenesis of the renal changes and their relationship to PSS remains obscure. Further studies should elucidate if both the dermal and the renal manifestations originate from a common immunologic reaction or the pathologic process in scleroderma

has initiated an (autologous) immune complex disease. We believe that kidney biopsies at early stages of nephropathic PSS would be useful to expand our knowledge on the pathogenesis of this disorder.

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