Clin. exp. Immunol. (1971) **8**, 761–774.

IMMUNOGLOBULIN CLASSES AND COMPLEMENT IN BIOPSIES OF NIGERIAN CHILDREN WITH THE NEPHROTIC SYNDROME

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(*Received* 29 *September* 1970)

SUMMARY

Pretreatment diagnostic renal biopsies from fifty Nigerian children with the nephrotic syndrome were investigated by immunofluorescence for immunoglobulin classes, complement and specific antigens. Nineteen of these were re-examined after an interval of 10–15 months. Forty-eight first biopsies were positive for bound γ-globulins, usually IgM and IgG but sometimes for one of these alone; IgA was not detected. Thirty-three were positive for bound complement (C_3 component). IgM was associated with granular deposits and complement, IgG with both granular and continuous deposits, the latter usually lacking complement. *Plasmodium malariae* antigen was found in nine of thirty-six cases examined; no *P. falciparum* or streptolysin-O antigens were observed. Immunoglobulins G and M were found in tubules in seventeen of the fifty patients, in five together with complement. *P. malariae* antigen was observed in tubules in eleven of thirty-six cases.

Repeat biopsies from four patients who had recovered were negative with all reagents. Patients on anti-malarial therapy only, and those responding poorly to steroids or cyclophosphamide, showed no significant change in glomerular fluorescence, but a higher incidence of tubular fluorescence was noted in second biopsies. In patients with a poor response to Imuran treatment the pattern of glomerular fluorescence changed from granular to diffuse and tubular staining was not observed. In some patients increased levels of antiglobulins (rheumatoid factor type) were detected in later sera. The nature of the bound immunoglobulins was confirmed by elution of complexes and immunodiffusion.

It is suggested that an antigen–antibody complex with *P. malariae* antigen can produce renal damage with liberation of autoantigens which have the capacity to initiate self-perpetuating autoimmune disease.

INTRODUCTION

Evidence has accumulated that immune complexes play an important role in the nephrotic

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syndrome of African children (Allison *et al.*, 1969; Ward & Kibukamusoke, 1969; Houba *et al.*, 1970; Adeniyi, Hendrickse & Houba, 1970). In all cases renal biopsies have shown immunoglobulins, usually together with complement, in the form of deposits in renal glomeruli. Epidemiological evidence (Giglioli, 1962a, b; Gilles & Hendrickse, 1963; Kibukamusoke & Hutt, 1967) has suggested that *P. malariae* infection is related to the nephrotic syndrome and evidence in support of this interpretation has been the finding of *P. malariae* antigen in the renal glomeruli of some cases (Allison *et al.*, 1969; Ward & Kibukamusoke, 1969; Houba *et al.*, 1970). However, many points about the natural history of the nephrotic syndrome of African children remain to be clarified.

We have, therefore, undertaken a detailed study of the distribution of different immunoglobulin classes, the C_3 component of complement (WHO, 1968), malarial antigens, fibrinogen and albumin in renal biopsies of affected children. During the course of this investigation it became apparent that the pathogenesis of the progressive disease might not be due only to malarial antigen, so that observations have been made of other antigens present in immune complexes localized in renal lesions of patients. Detailed characterization of these antigens will be presented elsewhere.

MATERIALS AND METHODS

Nephrotic children—first biopsies

Fifty Nigerian children with the characteristic picture of the nephrotic syndrome were studied. Massive albuminuria, striking hypoalbuminaemia with oedema, usually not associated with significant hypertension and/or azotaemia, were the clinical and laboratory criteria on which the diagnosis of the nephrotic syndrome was based. The majority of patients showed poorly selective proteinuria; thirty-one of them were males and nineteen females, their ages at the time of testing ranging from 2–12 years (mostly 3–8 years) and the duration of disease ranged from 1 week to more than 1 year. Twenty-six patients showed *P. malariae* in blood smears at the time of investigation, in four both *P. malariae* and *P. falciparum* were found, in three *P. falciparum* alone and in the remaining seventeen patients no parasites were detected. However, seven of the patients with negative blood smears were treated with antimalarials before admission.

Renal biopsies

Diagnostic percutaneous renal biopsies were performed by the method of Kark & Muehrcke (1954) in all children before treatment and were repeated in nineteen of them after a 10–15-month interval to assess progress. Biopsy specimens for fluorescence microscopy were frozen in liquid nitrogen and kept in a deep freezer at $-70°C$ until examined.

Drugs and dosage

Children with high to moderate selective indices of proteinuria were given prednisolone at a dosage of 2 mg/kg body weight daily in two or three divided doses for 4 weeks, which was then reduced to 1·5 mg/kg daily for 3 consecutive days a week for a further 4 weeks.

Others received cyclophosphamide in a single dose of 2–3 mg/kg per day for at least 12 weeks or azathioprine (Imuran) in a single dose of 2·5–3·0 mg/kg per day also for at least 12 weeks.

All patients received chloroquine followed by primaquine as antimalarials, standard diuretics in the presence of oedema and symptomatic treatment for other associated conditions.

Non-nephrotic controls

Postmortem kidney specimens (obtained soon after death) from two children (7 and 6 years old) who died as a result of injuries from road accidents and one biopsy specimen from post-mortem material (child 8 years old) were used as controls. None of these kidneys showed any abnormalities on microscopical examination.

Fluorescence microscopy

Cryostat sections (4–6 μ) were dried with a current of air at room temperature in an air-conditioned room for at least 15 min, washed in phosphate buffered saline (PBS) for 1½ hr (PBS changed three times), stained with appropriate dilutions of conjugates for 30 min in moist chambers and washed in PBS for 1½ hr (PBS changed three times). The preparations were mounted in a mixture of 9 parts of glycerol and 1 part of PBS and examined in a UV-microscope (Reichert, Zetophan-Binolux) using the HBO-200 light source and dark-field condenser. Kodak High Speed Ektachrome and Agfa-Colour were used for photomicrography.

The results were recorded according to the brightness of fluorescence as follows: negative (negative and/or weakly positive) or positive (positive to strongly positive) and the fluorescence was recorded according to pattern as granular, diffuse or continuous.

Conjugates

Selected commercial antisera (Institute of Vaccines and Sera, Czechoslovakia) to human γ-globulins, immunoglobulins (IgG, IgA and IgM), fibrinogen and albumin were conjugated with fluorescein isothiocyanate (FITC) according to the technique described by Holborow & Johnson (1967). Free fluorochrome was removed by passage of conjugates through G-25 Sephadex and/or by dialysis against PBS at 4°C. The specificity of conjugates was tested by immunoelectrophoresis, the fluorescein/protein ratio by spectrophotometry and the optimal dilution of conjugates fulfilled the criteria recommended by the International Workshops on Standardization in Immunofluorescence 1967 and 1969 (Holborow, 1970). FITC-conjugates (rabbit and goat) to the human C_3 component of complement (β-1-C) were kindly provided by Dr T. E. W. Feltkamp (Netherlands) and by Miles-Yeda Limited, Israel. Both conjugates developed a single precipitin line against whole human serum on immunoelectrophoresis but no lines against human IgG, IgA and IgM in immunodiffusion tests.

The preparation of FITC-conjugates to malarial antigens (*P. malariae* and *P. falciparum*) and testing of their specificity were described in our previous paper (Allison *et al.*, 1969). FITC-conjugate (horse) to antistreptolysin-O was kindly provided by Dr W. D. Brighton (National Institute for Medical Research, London).

Indirect fluorescence test—for antibodies against kidney tissue components

Cryostat sections from normal kidney were washed, overlayed with the test sample, washed and tested for immunoglobulins with appropriate conjugates.

Elution experiments

Kidney specimens were obtained at necropsy (soon after death) from three nephrotic patients and from two controls who died as a result of injuries.

The kidney specimens were immediately used for elution or stored in a deep freezer at −70°C. The cortex was separated from medulla, cut into small pieces, washed twice with PBS, pH 7·2, homogenized in PBS and centrifuged for 1 hour at 4°C (IEC, 1500 rev/min). The sediment (S_1) was washed seven times with PBS, pH 7·2, and eluted twice with 3–5 ml of citric acid, pH 2·5 or 3–5 ml of glycine buffer, pH 2·5. The supernatant was still turbid and was centrifuged for 6 hr at 4°C (IEC, 1500 rev/min). After this time the homogenate sedimented completely. The clear supernatant was removed and the sediment (S_2) washed and eluted as described above.

All the manipulations were performed at 4–10°C. The supernatants obtained during washing of homogenates were tested for immunoglobulins as described below. Most immunoglobulins were found in the supernatant after the first washing, the remainder in the second and third supernatants. No immunoglobulins were demonstrable in supernatants obtained after the fourth washing.

Eluates were neutralized to pH 7·0 and examined for immunoglobulins and other substances by Ouchterlony technique, immunoelectrophoresis and immunoplates. Whenever negative reactions were obtained the eluates were concentrated approximately four times by dialysis and tested again.

Antisera and other reagents

Antisera to human IgG, IgA and IgM from the Institute of Vaccines and Sera, Czechoslovakia, and kindly provided by Dr D. S. Rowe, WHO International Reference Centre for Immunoglobulins, Lausanne, Switzerland, were used for identification of immunoglobulins. On immunoelectrophoresis of whole human serum the antisera showed single precipitin lines for individual immunoglobulins.

Horse antiserum against whole human serum proteins and monovalent antisera against individual components of human serum were obtained from the Institute of Vaccines and Sera, Czechoslovakia, from Behringwerke AG, Germany and from the Central Laboratories of the Blood Transfusion Service, Amsterdam, Netherlands. Reagents (spleen extracts and serum antigen) and antiserum to *P. malariae* described in our previous paper (Allison *et al.*, 1969) were used for demonstration of *P. malariae* antigen–antibody complexes in the eluates.

Immunoglobulin levels. IgG, IgA and IgM were estimated by the technique of Fahey & McKelvey (1965) using the appropriate antisera and reference preparations for immunoglobulins kindly provided by Dr D. S. Rowe.

Immunodiffusion tests were done according to the technique of Ouchterlony (Weir, 1967).

Immunoelectrophoresis was carried out according to the method of Grabar & Burtin (1964).

Antiglobulins (rheumatoid-factor type) were estimated by the latex-fixation test (Houba & Allison, 1966). The International Reference Preparation of Rheumatoid Arthritis Serum was used for calculating antiglobulin activities which were expressed in terms of the International Unit of Rheumatoid Arthritis Serum per ml (Anderson *et al.*, 1970). It was found that the slide test (RA—Hyland) was not sensitive enough for this purpose.

OBSERVATIONS

FIRST BIOPSIES

(1) *Glomerular lesions*

(*a*) *γ-Globulins.* Immunoglobulin deposits were observed in forty-eight of fifty patients examined (the remaining two cases had only weak fluorescence). However, the pattern of fluorescence was variable. In twenty-one cases typical coarse and medium-sized granular deposits were observed along the capillary walls (Fig. 1). In all but one of the remaining cases a mixed pattern of fluorescence, previously described (Houba *et al.*, 1970), was found. In addition to granular deposits clearly resolved by the optical microscope there was apparently diffuse fluorescence along the glomerular capillary walls. In some cases (eighteen) the granular pattern of fluorescence, in others (eight) the diffuse pattern (Fig. 2), predominated. In one case there was typical continuous fluorescence along the capillary walls (Fig. 3).

(*b*) *Immunoglobulin classes (IgG, IgA and IgM).* The fluorescence pattern with specific conjugates (IgG and IgM) is summarized in Table 1. The majority of patients with exclusively or mostly granular fluorescence had both IgG and IgM, although in some only IgG or IgM was detected. In contrast, the one case with exclusively continuous fluorescence and the majority of those with mostly diffuse fluorescence showed only IgG. None of the patients showed IgA fluorescence in glomeruli.

(*c*) *Complement* (C_3 *component* = β-1-*C*). As summarized in Table 1, thirty-three of forty-eight patients showed definite fluorescence with anti-human complement conjugates. The two cases which were only weakly positive with anti-γ-globulin conjugates were definitely positive with anti-complement conjugates.

The fluorescence was quite intense but less widely distributed than immunoglobulin fluorescence in the same glomeruli; sometimes the complement fluorescence was focal or limited to parts of some glomeruli. Complement fluorescence was always granular, even in cases with predominantly diffuse immunoglobulin fluorescence. The latter showed, however, a much lower incidence of staining with anticomplement conjugates than those with granular or predominantly granular immunoglobulin fluorescence.

(*d*) *Antigens, fibrinogen and albumin. P. malariae* antigen was found in glomeruli of nine out of thirty-six cases examined. In most of these (six out of nine) the antigen was present together with granular deposits of immunoglobulins and complement; in three remaining cases, immunoglobulins (IgG and IgM) were demonstrated but not complement.

In contrast, *P. falciparum* antigen was not demonstrable in glomeruli of any nephrotic children, even though some showed these parasites in peripheral blood films.

The antistreptolysin-O conjugate gave no fluorescence in nephrotic patients, even though clear reactions were obtained with the same conjugate in patients with typical acute nephritis (Houba *et al.*, 1970).

Definite fluorescence with conjugates against albumin was found in four cases and against fibrinogen in 2 cases.

(2) *Tubular staining*

Immunoglobulins G and M were demonstrated in tubules in seventeen out of fifty first biopsies examined. The proportion was approximately the same whether the glomerular deposits were granular or predominantly diffuse. The tubular fluorescence was sometimes demonstrable in the form of coarse or medium sized granules between the nuclei and lumen

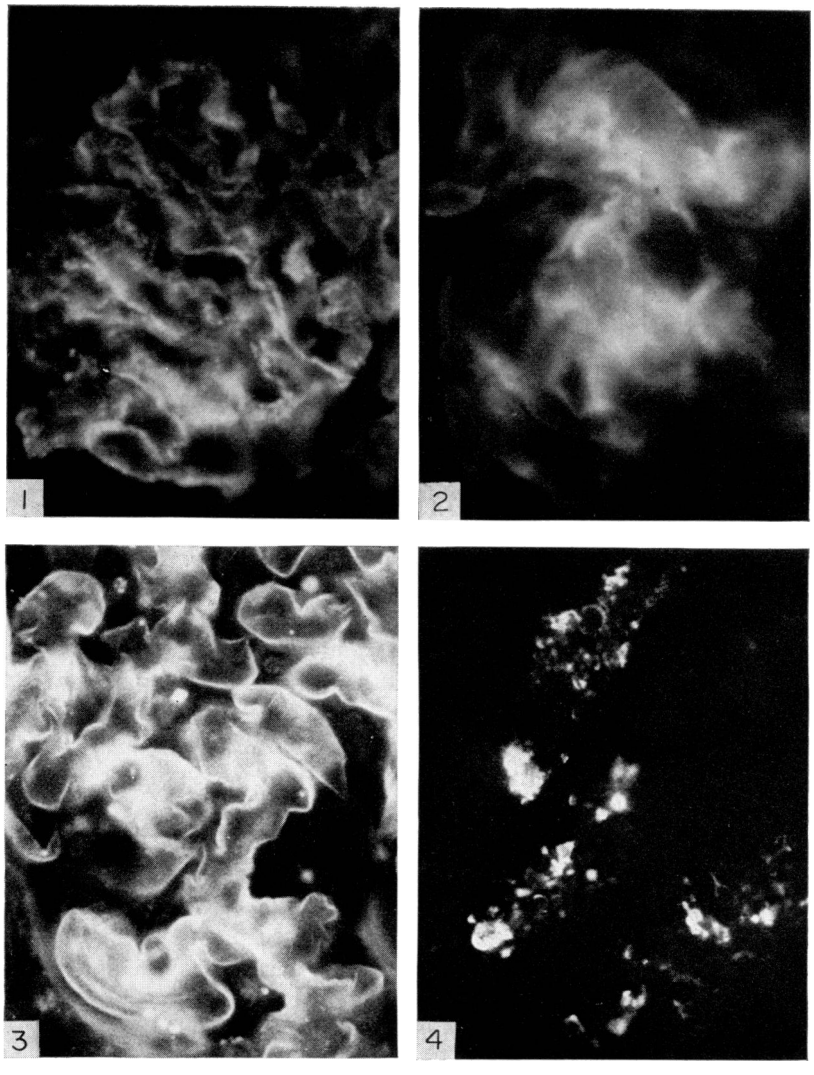

FIG. 1. Coarse and medium-sized granular fluorescence in a glomerulus. Anti-immunoglobulin conjugate.

FIG. 2. Predominantly diffuse glomerular fluorescence. Anti-immunoglobulin conjugate.

FIG. 3. Continuous, linear fluorescence along glomerular capillary walls. Anti-immunoglobulin conjugate.

FIG. 4. Discrete areas of fluorescence with conjugate against *P. malariae* in tubules.

but in other cases was apparently diffusely distributed throughout the cytoplasm of the tubular cells. Immunoglobulin A in tubules was negative except for two cases in which weakly positive reactions were obtained.

Complement in tubules was found in ten out of fifty patients. However, it was found in only five of the seventeen showing positive reactions for immunoglobulins.

P. malariae antigen was found in tubules of eleven of thirty-six cases examined, in two cases along with immunoglobulins and complement and in two cases with immunoglobulins only (Fig. 4). The remaining seven cases showing *P. malariae* antigen in tubules did not have demonstrable globulins or complement.

Again, *P. falciparum* antigen and/or streptolysin-O were not demonstrated in tubules of nephrotic patients. Conjugates against fibrinogen showed fluorescence in two cases, those against albumin in five cases.

TABLE 1. Patterns of fluorescence and distribution of immunoglobulins and C_3 component of complement in glomeruli of nephrotic children (cryostat sections from first biopsies)

Conjugates antihuman				
γ-Globulins		Immunoglobulins		C_3 component of complement
Pattern	Positive	Classes	Positive	Positive
Granular	21	G	6	4
		G, M	12	10
		M	3	3
Mixed, mostly granular	18	G	3	2
		G, M	14	11
		M	1	1
Mixed, mostly diffuse	8	G	7	1
		G, M	1	1
		M	—	—
Continuous	1	G	1	—
		G, M	—	—
		M	—	—
Total	48	—	48	33

REPEATED BIOPSIES

These are shown in Table 2, classified according to the therapy used.

In a few cases the first biopsy showed a typical granular or predominantly granular fluorescence pattern and there was a virtually complete recovery from symptoms after treatment with steroids (two cases), cyclophosphamide (one case) and azathioprine (one case). All the second biopsies (after recovery) showed no fluorescence in glomeruli and/or tubules with any of the conjugates used.

Patients receiving antimalarial therapy alone (first group) showed no clinical improvement or significant change in glomerular fluorescence intensity or pattern with conjugates against immunoglobulins G or M and complement (Fig. 5). Tubular staining for immunoglobulins G and M was often negative in first biopsies but consistently positive later; only three showed tubular staining with the anticomplement conjugate initially but all were positive later. One patient showed definite fluorescence with the antiIgA conjugate in a second biopsy.

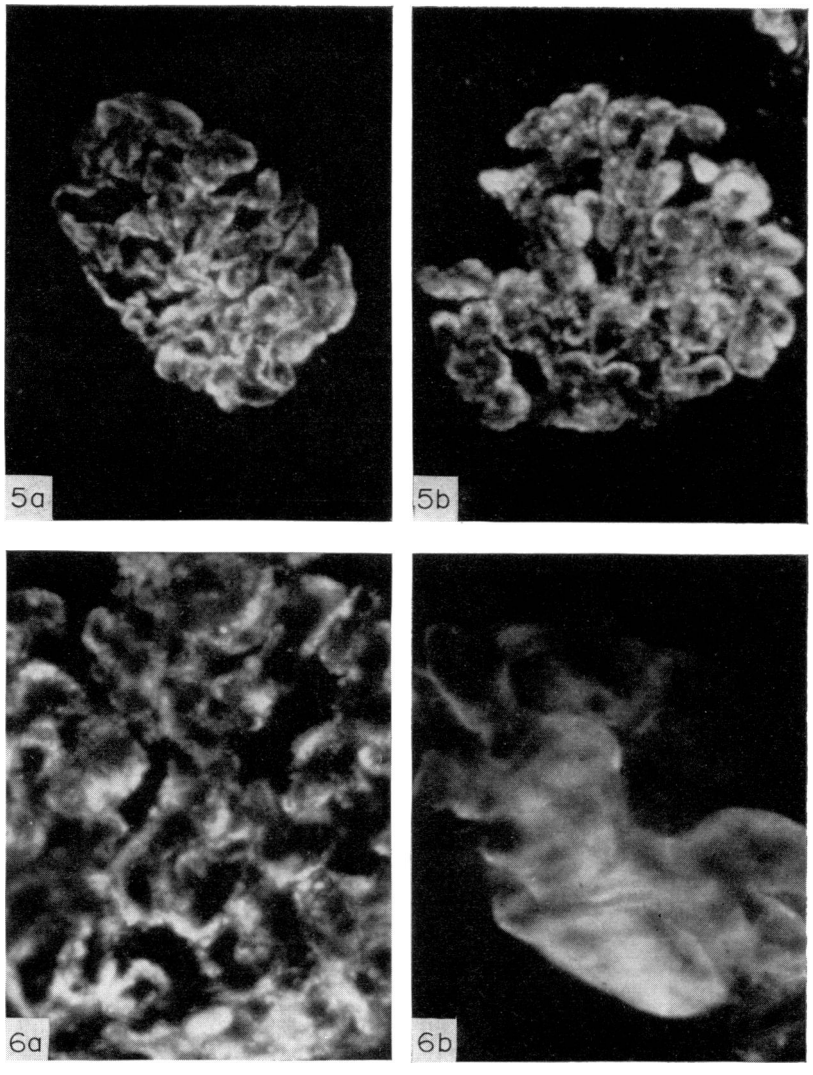

FIG. 5. Glomeruli of a patient before (a) and after (b) antimalarial treatment, showing unchanged granular fluorescence (anti-immunoglobulin conjugate).

FIG. 6. Glomeruli of a patient before (a) and after (b) treatment with azathioprine (anti-immunoglobulin conjugate). The granular immunoglobulin fluorescence pattern has changed to the diffuse.

The second and third groups of patients, responding poorly or not at all to steroids or cyclophosphamide, showed no significant changes in glomerular fluorescence. However, later intensification of tubular fluorescence, similar to that already described for the control group receiving antimalarials alone, was found.

A fourth group of patients was treated with azathioprine. The pattern of glomerular fluorescence with the immunoglobulin conjugates changed in three out of four patients who

showed a poor or no response to therapy. Initially, the pattern was granular or predominantly granular, whereas later it was more diffuse (Fig. 6). In contrast to the previous groups no fluorescence was observed in tubules after treatment. Only one patient (MA.2) with no clinical response to the therapy in this group revealed no change in pattern of glomerular fluorescence, that is, a picture similar to the previous groups. Moreover, this was the only patient from the whole series who showed definite fluorescence with the anti-IgA conjugate in both glomeruli and tubules on second biopsy. It should be noted that most glomeruli in the second biopsy specimen of this patient were badly damaged and degenerative changes in tubules were evident.

The incidence of positive fluorescence with other conjugates (against *P. malariae*, *P. falciparum*, streptolysin-O and fibrinogen) in second biopsies was similar to that in the first biopsies. Only antialbumin conjugates gave positive staining of tubules more frequently in second biopsies.

Other tests

Serum levels of immunoglobulins IgG, IgM and C_3 component of complement (β-1-C) were estimated in several nephrotic children.

There was a wide range in the values obtained, and the majority of patients showed relatively higher levels of IgM than IgG. However, there was no direct relationship between the serum levels of both immunoglobulins and/or the C_3 component of complement and their presence in tissue deposits and/or the pattern of fluorescence. Variations in immunoglobulin levels with regard to the duration or progress of the disease and also the effect of therapy will be analysed in detail in a separate study by one of us (A.A.).

Levels of antiglobulins (rheumatoid factor type) in serum were estimated in a few patients at the time of first and repeated biopsies. The findings are given in Table 2.

Elution experiments

Immunoglobulins were demonstrated by precipitation in the eluates of all the nephrotic kidneys but not in the eluates of control kidneys. It has been found that the eluates obtained from the first sediment (S_1) were mostly negative and the immunoglobulins were detected in the second sediment (S_2) obtained after prolonged centrifugation. The observations are summarized in Table 3.

All the eluates from nephrotic kidneys contained IgG but only one case (SJ) showed in addition IgM and antibody to *P. malariae*. The latter case was also strongly positive for IgG and IgM in immunofluorescence tests on cryostat sections—the pattern of fluorescence being typically granular. The staining of sections with anti-*P. falciparum* conjugates was negative but unfortunately the fluorescence test for *P. malariae* was not done for technical reasons. The remaining two cases (MR, MA) showed a mixed pattern of fluorescence with positive IgG but negative IgM in glomeruli; one patient (MR) had positive IgG and negative IgM in tubules, while the second patient (MA) showed positive staining for both immunoglobulins (G and M). Both patients (MR and MA) showed no reaction with conjugates against *P. malariae*.

All the eluates were tested for antibodies against kidney components using indirect immunofluorescence on cryostat sections from normal human kidney. No fluorescence was observed in glomeruli but weakly positive reactions were shown in tubules using eluates of kidneys from patients MR and MA.

TABLE 2. Repeated renal biopsies in nineteen children with nephrotic syndrome; comparison of immunofluorescence in glomeruli and tubules between first and second biopsies

Patients				First biopsy						Therapy		Second biopsy					
Initials	Sex	Age (years)	Duration of disease	Glomeruli			Tubules		RF	Used	Response	Glomeruli			Tubules		RF
				GG	Pattern	C_3	GG	C_3	In serum (IU/ml)			GG	Pattern	C_3	GG	C_3	In serum (IU/ml)
WY	M	5	5 m	+	M granular	+	−	−	nd	Antimalarials only	No	+	M granular	−	+	+	nd
KA	M	5	3 m	+	M granular	−	−	+	10		No	+	M granular	−	+	+	10
OO	F	5	1 m	+	M granular	+	−	+	nd		No	+	M granular	+	+	+	nd
FI	M	3	1 m	+	Granular	+	−	−	nd		No	+	Granular	+	+	+	nd
AA	M	8	3 m	+	Granular	+	−	+	2·5		No	+	Granular	+	+	+	2·5
RA	M	4	4 m	+	Granular	+	+	+	<2·0	Antimalarials and	V. good	−	—	−	−	−	20
FA	F	9	1 w	+	Granular	+	−	−	nd	steroids	V. good	−	—	−	−	−	nd
SS	F	7	5 m	+	Granular	+	+	+	nd		Poor	+	M granular	+	+	+	nd
BR	M	10	3 m	+	M granular	+	−	−	nd		Poor	+	M granular	−	+	+	nd
SE	M	6	3 w	+	M granular	+	−	−	<2·0		Fair	+	M granular	+	+	+	20
SK	M	10	2 m	+	M granular	+	−	−	nd	Antimalarials and	V. good	−	—	−	−	−	nd
AO	F	4	5 w	+	Granular	+	−	−	nd	cyclophosphamide	Poor	+	Granular	−	+	−	nd
WA	F	7	1 y	+	Granular	+	−	−	nd		No	+	Granular	+	+	−	20
MA_1	F	4	4 w	+	M granular	+	−	−	nd		Poor	+	Granular	+	+	+	nd
NA	M	2	2 w	+	Granular	+	−	−	nd	Antimalarials and	V. good	−	—	−	−	−	nd
MA_2	M	7	6 m	+	Granular	+	−	−	nd	Imuran	Poor	+	M granular	+	−	−	nd
WT	M	6	1 y	+	Granular	+	−	−	<2·0		No	+	Diffuse	+	−	−	20
MF	M	8	3 y	+	M granular	+	−	−	10		No	+	Diffuse	+	−	−	20
MA_3	M	6	7 m	+	M granular	−	+	−	nd		No	+	M granular	+	+	+	nd

Sex: M, male; F, female. Duration of disease: w, weeks; m, months; y, years. Fluorescence: +, positive; −, negative; nd, not done. GG: conjugate antihuman globulins. C_3: conjugate antihuman C_3 component of complement. Pattern: granular, typical granular only; M granular, mixed mostly granular; diffuse, mixed mostly diffuse. RF: antiglobulins of Rheumatoid Factor Type. IU/ml: International Unit of Rheumatoid Arthritis Serum per ml.

The eluates from kidneys of patients MR and MA showed in addition to IgG another two precipitin lines when tested against horse serum hyperimmunized against serum proteins. These precipitin lines were localized in the α_2–β_1 globulin region on immunoelectrophoresis but were not demonstrable with any of the monospecific antisera against serum proteins migrating in this region. The further characterization of this antigen, which may be relevant to the pathogenesis of the nephrotic syndrome, will be described elsewhere.

TABLE 3. Comparison of the incidence of immunoglobulins in tissue sections (immunofluorescence) and in eluates of kidneys (precipitation)

Patient	Immunofluorescence in cryostat sections				Eluates	
	Glomeruli		Tubules			
	IgG (Pattern)	IgM	IgG	IgM	IgG	IgM
SJ (Nephrotic)	++ (Granular)	++	−	−	+	+
MR (Nephrotic)	++ (Mostly granular)	−	−	−	+	−
MA (Nephrotic)	+ (Mostly diffuse)	−	+	++	+	−
Control	−	−	−	−	−	−
Control	−	−	−	−	−	−

DISCUSSION

The availability of repeated biopsies, which had been taken to evaluate the course of the nephrotic syndrome under different forms of treatment, provided a unique opportunity to follow the pattern of immunoglobulins, complement and antigen distribution at different times.

The first point to emerge from the study was that marked clinical improvement was well correlated with the disappearance of bound immunoglobulins and complement from the renal glomeruli. There was also a reversal toward relatively normal appearances in light microscopy (Edington *et al.*, in preparation) and in electron microscopy.

As previously reported (Allison *et al.*, 1969; Houba *et al.*, 1970), the immunoglobulins bound in the glomeruli were IgG and IgM. In contrast to the report of Ward & Kibukamusoke (1969), IgA in glomeruli was detected in only one biopsy taken after azathioprine treatment in which there was considerable glomerular damage. Hence the possibility of non-specific adsorption of conjugates to denatured materials cannot be excluded. We infer that IgA plays no significant part in the pathogenesis of the glomerular lesion in Nigerian nephrotic children.

IgM was always associated with granular deposits and was usually found together with complement. IgG was associated with both granular and diffuse patterns of fluorescence, but in the latter case complement was usually not demonstrable. This may be due to the

presence of an IgG subclass lacking complement-binding capacity, but further evidence is required to establish this point.

There was no clear relationship between the pattern of glomerular fluorescence and duration of disease, nor was the pattern significantly modified by treatment with antimalarials, steroids or cyclophosphamide. However, azathioprine treatment—in repeated biopsies from the small number of cases observed—produced a change from a typically or predominantly granular to a diffuse pattern of fluorescence in glomeruli.

The nature of the diffuse pattern of fluorescence remains to be elucidated. Possibly it corresponds to very fine localized deposits too small to be resolved by the optical microscope and similar to the deposits described by Weigle & Nakamura (1969). However, biopsy samples from patients with predominantly diffuse glomerular fluorescence did not show typical localized subepithelial deposits in electron micrographs, but rather irregular thickening and density of the basement membrane. In one case (patient MF, second biopsy after Imuran treatment, showing predominantly diffuse pattern of glomerular fluorescence) there was an accumulation of electron dense material in a subendothelial position, consistent with the possible production of antibody against glomerular basement membrane (Feldman, 1963). The possibility that azathioprine might induce an autoimmune lesion of this kind deserves consideration. Further evidence that nephrotic patients do not usually have antibody against glomerular basement membrane comes from our observations that eluates from three nephrotic kidneys failed to show any reaction with glomerular basement membrane. However, only one of them was obtained from a patient with predominantly diffuse glomerular fluorescence.

Prominent diffuse linear basement membrane and mesangial fluorescence was described in a few patients with severe post-streptococcal glomerulonephritis by Michael *et al.* (1966), but no such pattern was found in nephrotic children in East Africa (Ward & Kibukamusoke, 1969).

Only one of our nephrotic patients showed typical continuous or linear patterns of fluorescence, characteristic of antibodies against glomerular basement membrane as described in Goodpasture's syndrome and in experimental autoimmune glomerulonephritis (Unanue, Lerner & Dixon, 1968).

While the consistent presence of bound immunoglobulins and complement in substantial amounts in the glomeruli of nephrotic patients provides strong evidence in support of an immune complex aetiology, the nature of the antigens involved still requires careful consideration. *P. malariae* antigen was found in glomeruli of nine out of thirty-six first biopsies examined. This still appears to be a meaningful association when taken in conjunction with the epidemiology of the nephrotic syndrome and contrasted with the absence of other antigens such as *P. falciparum* and streptococcal antigens. However, an explanation is required for the absence of demonstrable antigen in the majority of cases. One possible explanation is parasite strain-specificity, but another is that *P. malariae* antigen–antibody complexes trigger a pathogenetic sequence in which autoantigens are later involved.

No antibodies against glomerular basement membrane were found but evidence implicating renal tubular antigens will be presented elsewhere. In this connection it is interesting that *P. malariae* antigen was quite frequently demonstrable in the tubules of nephrotic patients. Possibly the parasite antigen, or antigen–antibody complexes, can produce tubular damage with release of modified antigen leading to production of auto-antibodies. These might then give rise to progressive renal damage, as in the case of the self-perpetuating

nephritis in experimental animals elicited by immunization with renal tubular antigens (Heymann *et al.*, 1959, 1963, 1965; Dixon, Edgington & Lambert, 1968; Glassock *et al.*, 1968; Edgington, Glassock & Dixon, 1968; Edgington, Lee & Dixon, 1969; Barabas, Elson & Weir, 1969).

In a few of the patients increased levels of antiglobulins (Rheumatoid Factor Type) were found and in some individual cases a definite increase of these antiglobulins in serum over a period of about 1 year was demonstrated. This finding is in agreement with the widely accepted hypothesis that the antigenic stimulus for production of Rheumatoid Factor antiglobulins *in vivo* results from a change of the γ-globulin molecule after antigen–antibody combination, irrespective of the nature of the antigen involved (Henney, 1970). Hence, the soluble immune complexes circulating in nephrotic children before deposition in kidney may well be responsible for the production of these antiglobulins. The significance of the antiglobulins in nephrotic patients is not yet known; they may simply indicate the change of the γ-globulin molecule which becomes autoantigenic, or they may have functions (pathological or protective?) in the pathogenesis of the nephrotic syndrome similar to those described in patients with other immune complex diseases (Davis & Bollet, 1966; Lightfoot, Drusin & Christian, 1970; Goldberg & Barnett, 1970; Schultz, Kano & Milgrom, 1970).

ACKNOWLEDGMENTS

We are indebted to Mr John Ogingo for technical assistance and the Wellcome Trust for financial support.

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ABBREVIATION

FITC fluorescein isothiocyanate