Current Topics in Pathology

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Human Parathyroid Gland: A Freeze-Fracture and Thin Section Study

Malignant Nephrosclerosis in Patients with Hemolytic Uremic Syndrome (Primary Malignant Nephrosclerosis)

Liver Carcinogenesis in Rats After Aflatoxin B_1 Administration

Blastomycosis

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With 119 Figures

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Contents

Correlations Between Morphologic and Clinical Features in Idiopathic Perimembranous Glomerulonephritis

A Study on 403 Renal Biopsies of 367 Patients

H.V. GÄRTNER, T. WATANABE*, V. OTT, A. ADAM, A. BOHLE*, H.H. EDEL, R. KLUTHE, E. RENNER, F. SCHELER, R.M. SCHMÜLLING, H.-G. SIEBERTH

I. Introduction

Perimembranous glomerulonephritis (PGN), also called extramembranous glomerulonephritis (*Berger* et al., 1961; *Royer* et al., 1962; *Ducrot* et al., 1963), or epimembranous (*White*, 1969; *Seymour* et al., 1971), or membranous nephropathy (*Ehrenreich* and *Churg*, 1968; *Cameron*, 1970), is a morphologically well-defined disease occurring in both idiopathic and nonidiopathic forms. Together, they constitute about 9% of all inflammatory glomerular diseases in the patients examined by us over the past years (*Bohle* et al., 1974).

Little has been reported to date concerning the relationship between the morphologic and clinical findings of this disease entity (*Ehrenreich* and *Churg*, 1968; *Ducrot* et al., 1969; *Churg* and *Ehrenreich*, 1973; *Franklin* et al., 1973; *Gluck* et al., 1973; *Habib* et al., 1973; *Row* et al., 1975).

Our previous investigations of 100 patients with PGN (*Gärtner* et al., 1974) demonstrated along with *Ehrenreich* and *Churg* (1968), *Ducrot* et al. (1969), *Rosen* (1971), *Gluck* et al., (1973), that PGN may occur at any age, more often, however, between 31 and 60, and nearly twice as frequently in males as in females (*Pollak* et al., 1968; *Ducrot* et al., 1969; *Habib* et al., 1971; *Rosen*, 1971). The disease begins insidiously with no characteristic history of illnesses. Anamnestically dominant initial symptoms are edema or edema and proteinuria (*Ehrenreich* and *Churg*, 1968; *Pollak* et al., 1968; *Ducrot* et al., 1969; *Foreland* et al., 1969; *Gluck* et al., 1973). The disease climaxes in a fully developed nephrotic syndrome (*Ehrenreich* and *Churg*, 1968; *Pollak* et al., 1968; *Habib*, 1970) combined in about half of the patients with minor hypertension and hematuria. In accordance with *Ehrenreich*

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and *Churg*, we subdivided the developing progressive changes in the glomerular basement membrane into four stages to demonstrate, in agreement with these authors, as well as with *Hatta* (1972), that the degree of transformation of the basement membrane was dependent on the duration of the disease. The results of the morphometric examinations confirm, in contrast to the opinion of other authors (*Cameron*, 1970; *Churg* et al., 1970; *Habib*, 1970; *Murphy* et al., 1973) that PGN belongs to the proliferative glomerular disorders. The prognosis of PGN is unfavorable, regardless of the therapy administered. Till now, it remains to be answered whether the specific morphologic changes, classified by *Ehrenreich* and *Churg*, into four stages, are paralleled by specific clinical symptoms and whether an alteration in these symptoms correlates with changes in morphologic structures, thus allowing a prognosis on the basis of the morphologic picture. Furthermore, it is unknown whether nonspecific morphologic changes, in addition to the typical progressive basement membrane lesions, have an influence on the course and prognosis of the disease, and whether a patient's sex affects the features and course of the disease. To examine the correlations between morphologic findings and clinical features at the time of biopsy, and to determine the further morphologic and clinical course of PGN by serial renal biopsies, we have evaluated our experience of the past ten years with 367 patients with idiopathic PGN.

II. Material and Methods

Renal biopsies of 439 patients with PGN, diagnosed between 1964 and 1974, served as the starting point for our study. Because only patients with idiopathic PGN should be considered, 72 patients with nonidiopathic PGN were eliminated, thus providing a total of 367 patients for our examination. Nonidiopathic PGN occurred either in connection with drug therapy (D-penicillamine, gold, tridione), or SLE, diabetes mellitus, or a malignant tumor. D-penicillamine-induced PGN has been reported on elsewhere (*Gärtner* et al., 1975).

Of the 367 patients 38 were serial biopsied, 31 of them twice, 5 three times, and, in two cases, four biopsies were performed, so that a total of 414 biopsies were available for the following study. Because, for technical reasons, the silver staining did not succeed in 11 cases, the morphologic and clinical analysis could be performed on only 403 renal biopsies, taking into account 13 autopsies—55 cases were additionally examined immunohistologically in 15 cases electron-microscopically.

Microscopic examinations were performed on PAS-stained sections, embedded in paraffin, and on Movat silver-stained semithin sections (1 μ thick) to characterize the typically occurring morphologic changes in the glomerular basement membrane in PGN, according to the classification of *Ehrenreich* and *Churg*. FITC-labeled antisera were supplied by Behring for the immunohistologic studies (antihuman-IgG, IgM, IgA, IgD, IgE, -HAA, albumin, and complement). Electron-microscopic examinations were performed in paraffin-embedded tissues, inlaid in plexiglass.

In evaluating the clinical data, the following parameters were considered:

1. Age and sex
2. History of illnesses
3. First subjective and objective symptoms of the disease
4. Period of observation, i.e., the time between the appearance of the first renal symptoms and the renal biopsy, subsequently referred to as duration of the disease

5. Clinical symptoms and laboratory findings at the time of the biopsy

The morphologic changes in each renal biopsy were simultaneously classified into stages without knowledge of the clinical data, thus allowing the following correlations between the morphology and clinical features to be examined:

1. Stage, age, and sex
2. Stage and duration of the disease
3. Stage and proteinuria
4. Stage and nephrotic syndrome
5. Stage, blood pressure, serum creatinine, and creatinine clearance
6. Stage and clinical course of the disease
7. Stage and therapy.

In addition, the morphologic and clinical results from 38 cases with serial renal biopsies were analyzed.

The nephrotic syndrome was defined as follows–proteinuria more than 3 g/24 h, total serum protein less than 6 g/100 ml, albuminemia less than 3 g/100 ml. Edema and hypercholesterinemia were not obligatory. A blood pressure with a systolic value greater than 140 mm Hg and a diastolic value more than 90 mm Hg was considered to be elevated. Creatinine clearance values less than 80 ml/min/1.73 m^2 were regarded as being mildly limited and less than 40 ml/min/1.73 m^2 as markedly limited. Serum creatinine values up to 1.2 mg% were normal. The erythrocyturia was classified in three degrees of severity:

1. 3-10 erythrocytes/field of vision = mild (+)
2. 11-20 erythrocytes/field of vision = medium (++)
3. more than 20 erythrocytes/field of vision = severe (+++)

This classification of the erythrocyturia refers to the sediment obtained from freshly collected urine samples centrifuged under standard conditions and examined under 40-fold magnification.

The evaluation of clinical criteria of the clinical course of the disease was based on the proposals of *Renner* et al. (1969)–an improvement or deterioration occurred by an alteration in the creatinine clearance values of more than 15 ml/min, in the proteinuria by more than 50% of the starting value, in the total serum protein by more than 1 g/100 ml, in arterial systolic blood pressure by more than 20 mmHg, in diastolic blood pressure by more than 10 mm Hg, and in increase or decrease in eryhtrocyturia. Differences in the mean values were considered significant if the level of significance in the F-test according to R.A. Fischer was $2\,P \leqq 0.05$.

III. Results

1. Clinical Findings

a) Age, Sex (Fig. 1): Idiopathic PGN occurs in every age group, ranging between 1 and 75, with a peak between 31 and 50 years. The average age is 39. Males are afflicted twice as frequently as females (67% males : 33% females).

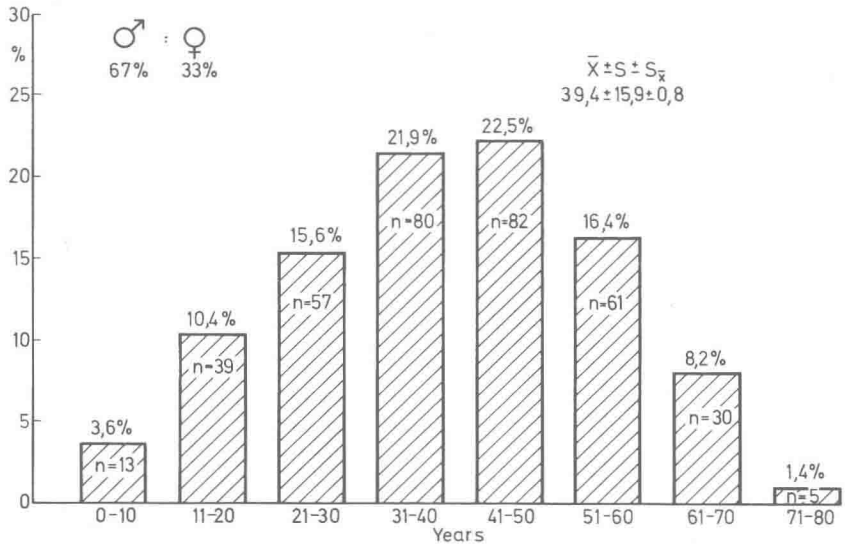

Fig. 1. Distribution of sex and age of 367 patients with idiopathic PGN at time of first renal biopsy

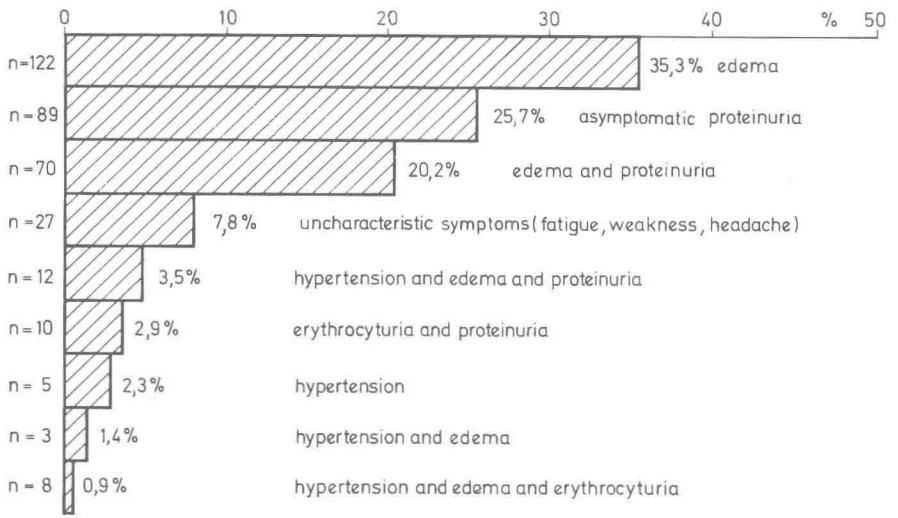

Fig. 2. First subjective and/or objective symptoms of the disease in 367 patients with idiopathic PGN

b) Previous Diseases: Tonsillitis (37%), bacterial and viral infections (22%), as well as scarlet fever and rheumatic fever (4%), are most often revealed in the past history. Approximately a third of the recorded previous diseases are heterogeneous disorders which some-

times occurred so long ago that no connection with the PGN can be established. In 15% of the cases, there is no history of illnesses.

c) Initial Subjective and/or Objective Clinical Symptoms of the Disease (Fig. 2). The first clinical symptoms, associated with an acute nephrotic syndrome, are most often anamnestically reported as edema, either alone or combined with proteinuria, if a medical examination took place at that time. In a quarter of the patients, an asymptomatic proteinuria characterized the insidious beginning of the disease.

Hypertension or hypertension with proteinuria and erythrocyturia, as well as uncharacteristic symptoms as fatigue and weakness, are rarely reported as initial symptoms.

d) Duration of the Disease, or the period of observation from the first appearance of subjective and/or objective symptoms to the renal biopsy is an average of 21 months. Nearly two-thirds of the patients are biopsied in the first year.

e) Clinical Findings at the Time of the Biopsy (Table 1). This table shows that a nephrotic syndrome exists in 70% of the patients at the time of biopsy (average proteinuria 6.9 g/24 h).

Table 1. Clinical findings of 367 patients with idiopathic PGN at time of first renal biopsy

Proteinuria (g/day)	Nephrotic syndrome	Erythrocyturia
1- 3 : 33%	70%	No 31%
4- 6 : 32%	No nephrotic syndrome at any time (18%)	Mild (+) 53%
7-12 : 25%		Medium (++) 12%
13-50 : 10%	Initial nephrotic syndrome, later disappearing (12%)	Severe (+++) 4%
$\bar{x} \pm s \pm s_{\bar{x}}$:		Macrohematuria –
6.9 ± 5.8 ± 0.3		

Blood pressure (mm Hg)	Creatinine clearance (ml/min/1.73 m^2)	Serum creatinine (mg %)
Systolic blood pressure	< 120 59%	to 1,2 : 70%
$\bar{x} \pm s \pm s_{\bar{x}}$	80 - 120	1.3 - 1.4 : 13%
154 ± 22 ± 1	40 - 79 23%	1.5 - 5 : 14%
Diastolic blood pressure	< 40 6%	5.1 - 30 : 3%
$\bar{x} \pm s \pm s_{\bar{x}}$	Not determined 12%	$\bar{x} \pm s \pm s_{\bar{x}}$
95 ± 12 ± 1		1.6 ± 2.5 ± 0.13

$\bar{x}$ = means; s = SD (standard deviation); $s_{\bar{x}}$ = SEM (standard error of the mean)

Of the 30% who had no nephrotic syndrome at the time of the biopsy, 12% showed a nephrotic syndrome at the beginning of the disease which then disappeared with or without therapy as the disease progressed, and 18% showed no nephrotic syndrome at any time. Most of the patients (53%) had only a mild erythrocyturia, if any at all. Severe erythrocyturia was observed in only a few patients and a macrohematuria was not observed.

The average systolic, as well as diastolic, blood pressure at the time of the biopsy was slightly elevated. Most patients had normal serum creatinine (70%) and creatinine clearance values (59%).

2. Morphologic Findings

The progressive morphologic changes, developing during the course of PGN, were classified on silver-stained semithin sections, according to *Ehrenreich* and *Churg*, into four stages. We introduced a fifth stage, designated as a reparation stage, in contrast to the fifth stage of *Ehrenreich* and *Churg*, which is defined as an end stage. The following findings could therefore be reported:

1. The semithin sections of 85 biopsies (21% of the total number) exhibit alterations representing stage I, as described by *Ehrenreich* and *Churg*, with scattered, distinctly vis-

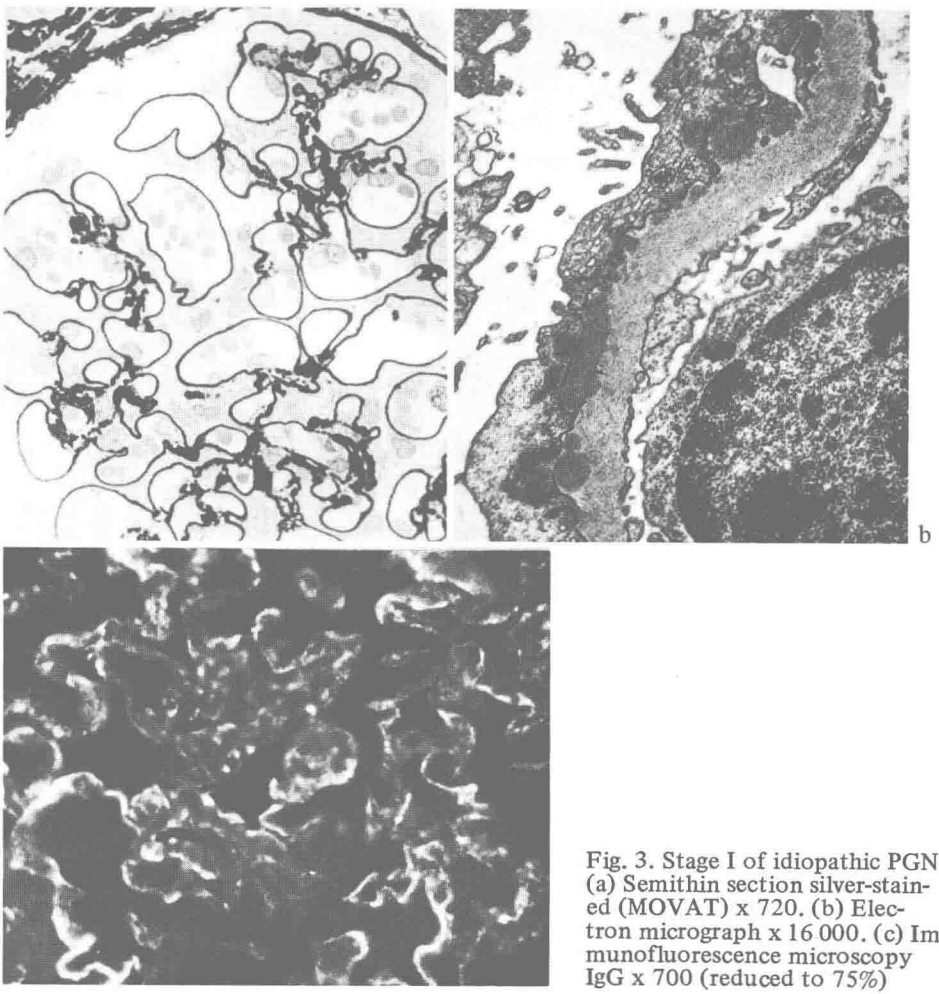

Fig. 3. Stage I of idiopathic PGN. (a) Semithin section silver-stained (MOVAT) x 720. (b) Electron micrograph x 16 000. (c) Immunofluorescence microscopy IgG x 700 (reduced to 75%)

ible projections (spikes) on the epithelial side of the glomerular basement membrane (Fig. 3a). Electron-microscopic examinations of five biopsies in this stage showed several subepithelial electron-dense deposits (Fig. 3b). The foot processes over the deposits were always fused and swollen or distorted. In 19 cases, the alterations observed microscopically were so minor that a final diagnosis could be established only by immunohistologic methods, showing a fine granular fluorescence along the glomerular capillary wall (Fig. 3c). As Table 2 indicates, IgG was deposited in combination with β_{1C} in all cases, with two exceptions. Six cases showed an additional deposition of IgM, four cases of IgA.

Table 2. Immunohistologic findings in 55 cases of PGN

Stage Detection of	Stage I n = 19	Stage II n = 22	Stage III n = 8	Stage IV n = 4	Stage V n = 2
IgG	19/19	21/22	7/8	4/4	1/2
IgM	6/19	8/22	5/8	1/4	∅
IgA	4/19	6/22	3/8	3/4	∅
β_{1C}	17/19	22/22	8/8	3/4	∅
Fibrinogen	∅	1/22	1/8	∅	∅
Negative	∅	∅			1/2
Special findings			3 cases: IgG, IgM, β_{1C}	1 case: 1 IgG, IgA	
			2 cases: 1 IgG, IgM, IgA, β_{1C}		
			1 case: IgA, β_{1C}		

2. In 170 biopsies (42% of the total), the typical morphologic alterations of stage II were fully developed and, often, the entire glomerular capillary walls were covered with numerous brown deposits, dispersed with argyrophilic spikes on the epithelial side of the tooth-like basement membrane (Fig. 4a). In three cases examined electron-microscopically (Fig. 4b), newly formed basement membrane material was found between the subepithelial electron-dense deposits, some of which were already incorporated into the basement membrane. The immunohistologic picture of 22 stage II cases (Fig. 4c) showed an intensive, granular, pearl necklace fluorescence along the capillary wall with evidence of IgG + β_{1C} or IgM, respectively, IgA and β_{1C}, or several immunoglobulins + β_{1C} (Table 2). The most frequent deposition in this stage was also IgG and β_{1C}. IgG was found either as sole immunoglobulin or in combination with others.

3. In 82 biopsies (20% of the total), stage III was observed, characterized by an extensive incorporation of brown deposits by adjacent spikes (Fig. 5a). Three cases examined electron-microscopically showed numerous electron-dense deposits incorporated into the basement membrane (Fig. 5b). In addition, isolated electron-lucent areas within the basement membrane were found. Immunohistologic investigations of eight cases (Fig. 5c) indicated an intense, coarse, pearl necklace fluorescence along the capillary wall. A deposition of IgG

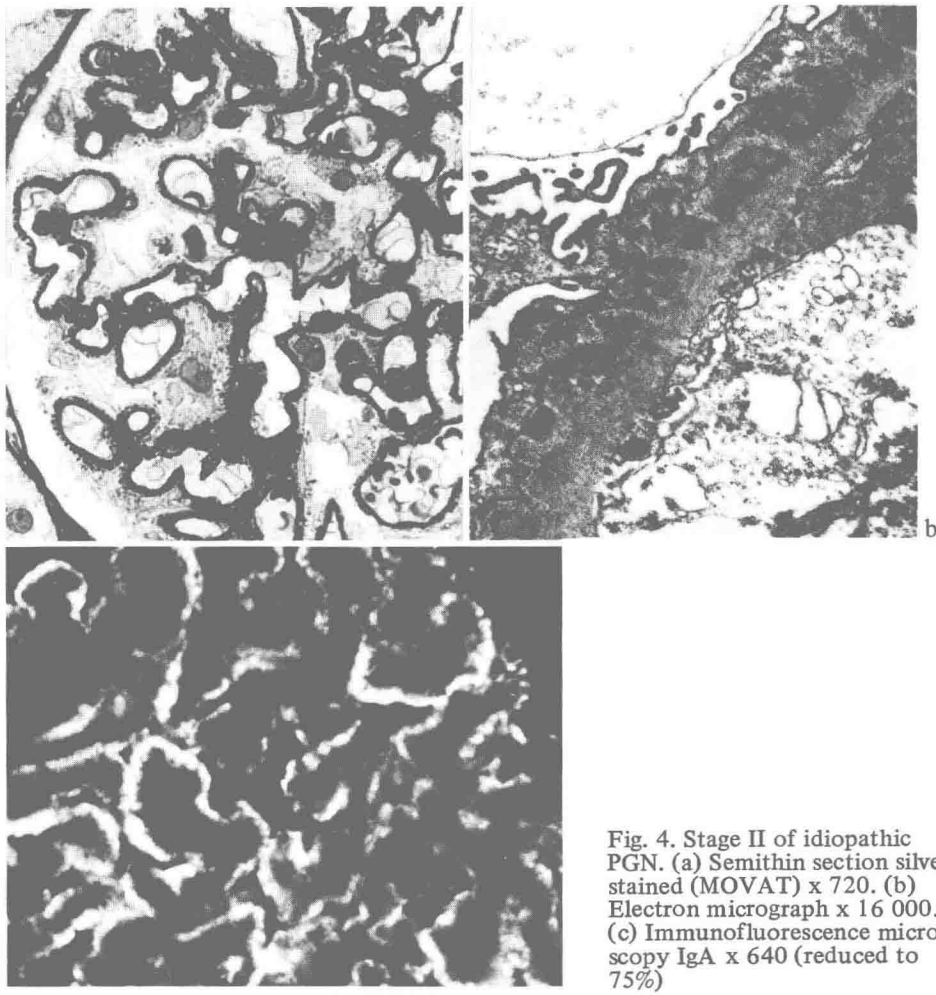

Fig. 4. Stage II of idiopathic PGN. (a) Semithin section silver-stained (MOVAT) x 720. (b) Electron micrograph x 16 000. (c) Immunofluorescence microscopy IgA x 640 (reduced to 75%)

and β_{1C} was found in most cases, however, an additional deposition of IgM was detected more often than in stages I and II.

4. In 45 biopsies (12% of the total), alterations representing stage IV were observed, characterized by a chain-like transformation of the basement membrane which appeared split and contained oval holes (Fig. 6a). Only a few isolated brown deposits, considerably smaller than those in stage III could still be found within the chain-like structure. In addition, six cases showed along with the alterations of stage IV, a segmental accumulation of deposits and some spikes, characteristic of stage II. In the two cases examined electron-microscopically, the chain-like parts of the basement membrane corresponded to numerous electron-lucent vacuolated areas, often located near the endothelial part of the basement membrane (Fig. 6b). The four cases examined immunohistologically demonstrated a coarse granular fluorescence along the capillary wall, subjectively less intensive and irregular than in the stage described above (Fig. 6c).

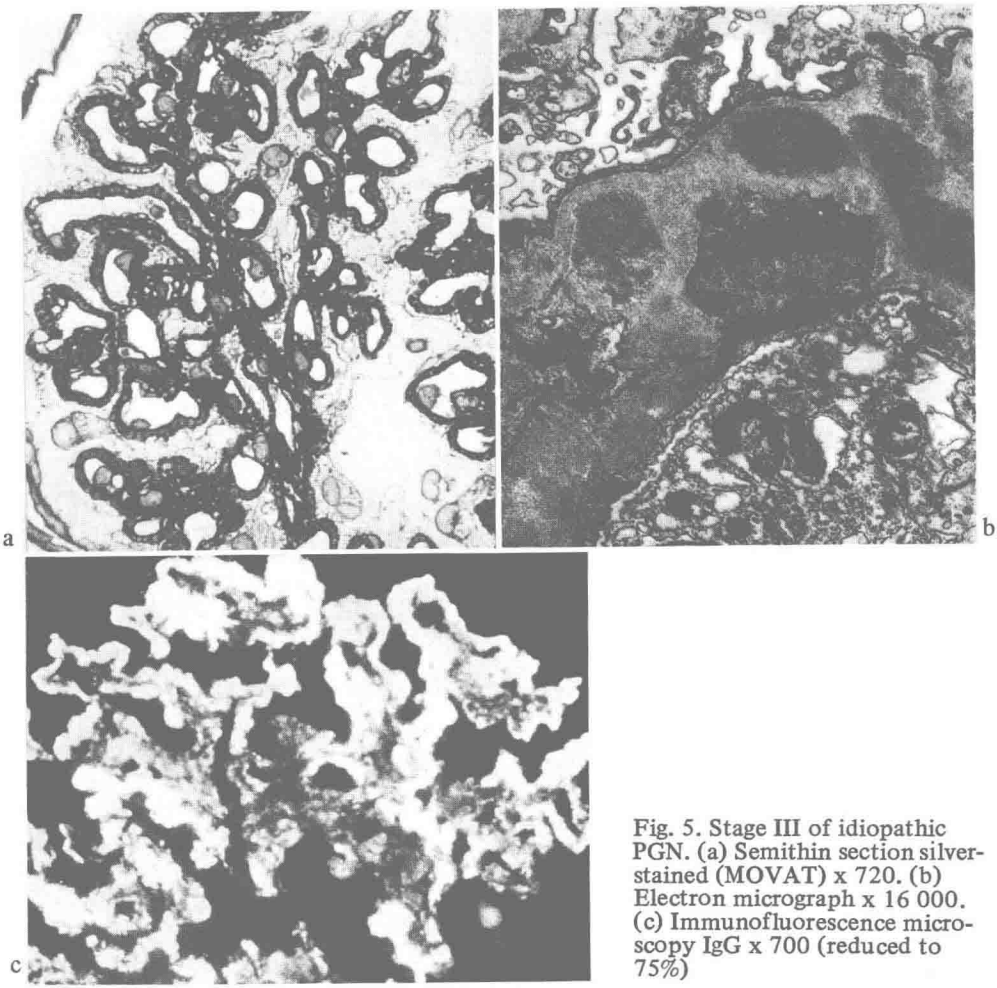

Fig. 5. Stage III of idiopathic PGN. (a) Semithin section silver-stained (MOVAT) x 720. (b) Electron micrograph x 16 000. (c) Immunofluorescence microscopy IgG x 700 (reduced to 75%)

In one case, complement was no longer detected (Table 2). In all other cases, it was present along with IgG.

5. In 21 cases (4% of the total biopsies), the alterations, designated by us for stage V, were found. In contrast to the end stage V of *Ehrenreich* and *Churg*, we considered it a reparation stage when an extensive normalization of the basement membrane occurs. In the semithin section, the basement membrane appeared to be very delicate and still only partially thickened or chain-like transformed (Fig. 7a).

The electronmicroscopic pictures of the four cases examined again showed electron-lucent areas of various sizes along with entirely normal basement membrane parts (Fig. 7b). Electron-dense deposits were no longer visible. Of the two cases examined immunohistologically, one showed negative results, whereas the presence of IgG in the area of the capillary wall could be demonstrated in the other but without complement (Table 2).

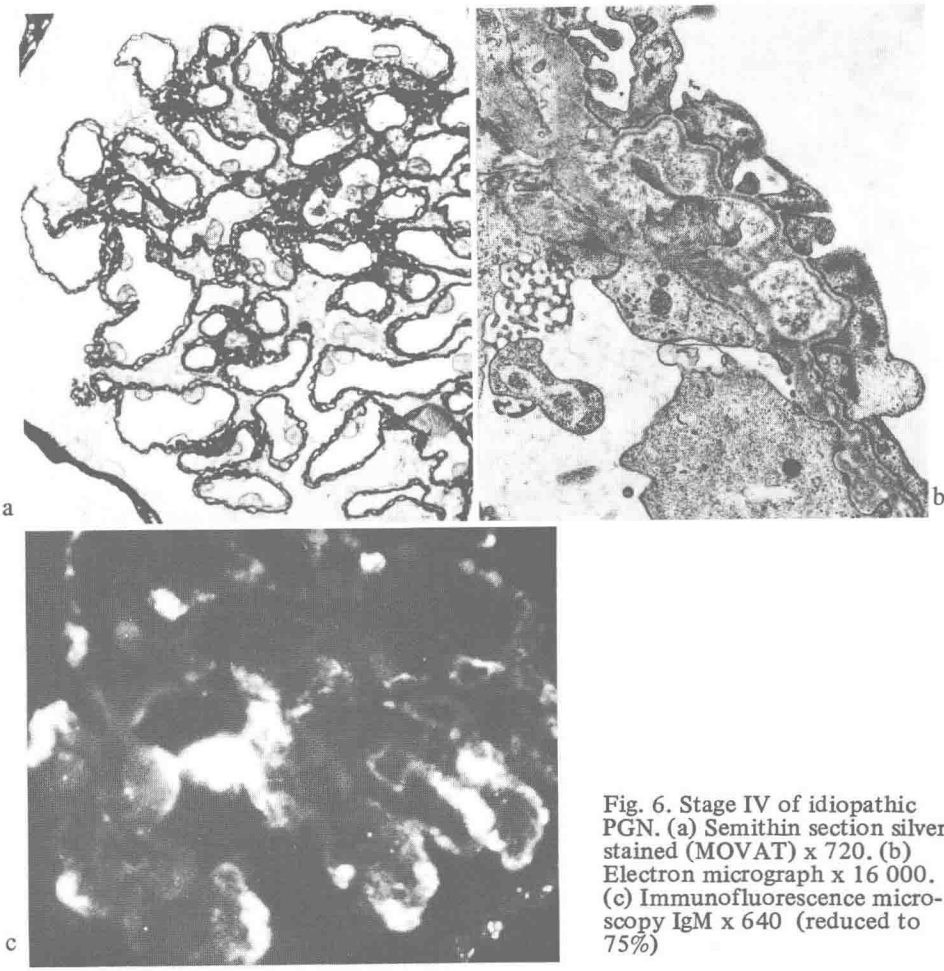

Fig. 6. Stage IV of idiopathic PGN. (a) Semithin section silver-stained (MOVAT) x 720. (b) Electron micrograph x 16 000. (c) Immunofluorescence microscopy IgM x 640 (reduced to 75%)

3. Correlations Between the Morphologic Stage and Clinical Parameters (Fig. 8)

1. Figure 8 illustrates the correlation between the morphologic stages of PGN and the duration of the disease. The duration of the disease (period between the first appearance of clinical symptoms and the renal biopsy) was shortest in stages I and II and longest in stages IV and V. It can, therefore, be concluded that the morphologic changes in the basement membrane were dependent upon the duration of the disease and that the degree of basement membrane transformation increased the longer the disease persisted.

2. Between the morphologic stages of PGN and the degree of proteinuria, as well as the appearance of the nephrotic syndrome, exists an inverse relationship (Fig. 9). Whereas, in stages I and II, the highest proteinuria values were observed, in stage V, only a minor proteinuria existed. The frequency of the nephrotic syndrome declined correspondingly with the decrease in proteinuria in relation to the duration of the disease, and to the degree of