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The Histopathology of the Kidney in Toxaemia. Serial Renal Biopsies During Pregnancy, Puerperium and Several Years Postpartum

Light and Electron Microscopic and Immunofluorescent Studies

ERNICO FIASCHI and REMO NACCARATO
Institute of Medical Pathology University of Padua, Italy

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Die Histopathologie der Niere in Toxämie, Nierenbiopsien während der Schwangerschaft, sofort und nach verschiedenen Jahren nach der Entbindung

Mikroskopische, elektronenoptische und Immunofluorescenzuntersuchungen

Zusammenfassung. 47 Nierenbiopsien von 38 Frauen mit Eklampsie und Präeklampsie werden mikroskopisch und elektronenoptisch untersucht. Die Biopsien stammen: 1. aus den letzten Monaten der Schwangerschaft, 2. 1 Std bis 1 Monat nach der Entbindung, 3. aus einer Nachkontrollperiode bis 4 Jahre nach der Entbindung.

Die Nierenveränderungen stimmen bei eklamptischen und präeklamptischen Patientinnen völlig überein. Die schwersten Veränderungen finden sich in den ersten Tagen nach der Entbindung. Bei allen Patientinnen können glomeruläre Schäden verschiedener Art und verschiedener Ausdehnung während Monaten und selbst noch nach Jahren nach der Toxämie beobachtet werden. In 16 Fällen wurden Immununtersuchungen durchgeführt mit fluorescierendem Isozyanat. Positive Reaktionen wurden beobachtet mit antihumanem 7 S-Globulin und antihumanem Fibrinogen. Aus den Untersuchungen geht hervor, daß die präeklamptischen und eklamptischen Nierenschäden sich, bis 4 Jahre nach der Toxämie, nicht vollständig zurückbilden. Die Ergebnisse der Immunfluorescenzuntersuchungen lassen noch keine bindenden Schlüsse zu.

Summary. Light and electron microscopic studies were carried out on 47 renal biopsies from 38 women with eclampsia and pre-eclampsia: (1) in the final months of pregnancy; (2) 1 hour to 1 month following delivery; and, (3) over an extended period up to 4 years postpartum. No distinction could be made between renal morphological alterations in eclamptic and pre-eclamptic patients. The most prominent lesions were found in the first days of the puerperium. In all the patients glomerular lesions varying in degree and distribution were observed months and even years following toxaemia.

In addition, immunofluorescent studies were performed on 16 specimens using various antisera labelled with fluorescein isothiocyanate. Positive reactions were observed with antihuman 7 S globulin and antihuman fibrinogen antisera.

Our findings have shown that renal lesions of toxaemia do not completely regress even after periods of up to 4 years. The immunofluorescent results were too limited for drawing definitive conclusions.

Renal lesions of eclampsia and pre-eclampsia have been studied by means of percutaneous biopsies and electron microscopy by numerous authors (FARQUHAR, 1959; SPARGO, 1959; FIASCHI, 1962, 1962; MAUTNER, 1962; WAKAMORI, 1962; DEROT, 1963; VASSALLI, 1963; NACCARATO, 1965). — The studies have made a significant contribution to the description and definition of the renal alterations characteristic of eclampsia and pre-eclampsia. Very few studies have

been devoted however to a long-term follow up of these changes to asses their evolution after delivery.

Coppitz (1957), Dieckmann (1958), Pollak (1960), and McCartney (1964) have reported that renal lesions in eclamptic patients regress completely after delivery. A similar finding in toxaemic patients was described by Merriel (1964). However; Donato (1957) and von Graf (1964) held that, on the basis of renal functions studies, renal damage in eclampsia may become permanent. Later electron microscopic studies confirmed that assumption. Thus, Hopper (1961) reported that in 3 exlamptic patients renal endothelial lesions were still evident 5 weeks postpartum. In 2 eclamptic patients, Mautner (1962) observed renal endothelial and mesangial alterations 5 months and 2 years after delivery. Recently, Dennis (1963) has studied, by light microscopy, renal specimens obtained from 9 exlamptic patients 6 months postpartum. Four specimens histologically were essentially normal. Arteriolar sclerosis was observed in 3 and in the remaining 2 the characteristic lesions of eclampsia were associated with arteriolar sclerosis.

In light of these conflicting observations, we decided to carry out histological investigations in late pregnancy and in the puerperium, with particular emphasis on serial studies.

Material and Methods

Our studies were carried out on 47 renal tissue specimens from 38 women[1]. The subjects were carefully selected to exclude patients suffering from any other type of kidney disease, hypertension, or diabetes mellitus. Repeat biopsies were performed in 7 patients and 2 of these patients underwent three biopsies each. The specimens may be divided into the following 3 groups: (1) 18 obtained in the final months of pregnancy of which 10 were eclamptic and 8 pre-eclamptic patients; (2) 12 obtained during the puerperium from 1 hour to 1 month following delivery; (3) obtained during a year period following eclampsia or pre-eclampsia.

Biopsies were performed with the usual technique of Kark and Pirani in the followup studies during the puerperium and then after, but the sitting position had to be adopted during pregnancy. The specimens were divided into two parts immediately following biopsy. One part was fixed in Bouin's solution, dehydrated, set in paraffin, cut in 4 to 5 μ sections, stained with hematoxylin and eosin or Schiff's reagent, and observed with a Leitz microscope. The other was fixed in 2% buffered osmic acid (Palade) for electron microscopy. After dehydration, it was embedded in butlymethylmethacrylate, vestopal or araldite. Sections 1 μ thick were cut on Porter-Blum microtomes, MT_1 and MT_2, using a glass knife, and were stained with Schiff's reagent or with silver methenamine. Ultra-thin sections, 100—200 Å, were also cut with the same microtomes, deposited on metal grids, stained with $KMnO_4$, phosphotungstic acid, $Pb(OH)_2$, or uranylacetate, and observed with a Siemens Elmiskop I electron microscope. Only biopsies containing more than 7 glomeruli were accepted for study.

Immunofluorescent studies were performed on renal tissue specimens from 16 patients (8 pregnant, 3 during the puerperium and the remaining 5 during the first 3 years postpartum). The tissues were stored at —50° C until used. Sections 3—4 μ thick were cut a cryostat, fixed in acetone. washed in buffered solutions, and then covered with fluorescein conjugated antisera, antihuman 7 S globulin and antihuman fibrinogen. Antihuman B_1C complement and Anti MA_{12} streptococci, and anti $T_{12}M$ antigen streptococci was used in one biopsy[2].

[1] The clinical details concerning the patients discussed in this report are available in the archives of the Gynecology Clinics at the Universities of Cagliari (30 observations) and Padova (8 observations). The morphological (optical, electron and immunohistological) findings are available in the archives of the Institute of Medical Pathology at the University of Padova.

[2] We wish to thank Professor B. C. Seegal, Department of Microbiology, Columbia University, New York City, and Professor B. Pernis, Director, Department of Medicina del Lavoro, University of Genoa, for their kind assistance in the immunhistological studies.

Results

The results of our studies can be summarized as follows: (1) The most characteristic alterations in toxaemia involve the glomeruli and, in particular, the glomerular endothelium (proliferation and swelling of the endothelial cells; thickening of the basement membranes; obstruction of several capillaries). No distinction could be made between eclamptic and pre-eclamptic patients (Figs. 1, 2, 3); (2) The most prominent renal lesions are found in the first days of the puerperium, regressing at least partially, towards the second week. Cytoplasmic

Table. *Cases studied with immunofluorescent techniques*

Patient	Age (in years)	Period of pregnancy	Syndrome	Delivery	Anti-human 7SG	Anti-human fibrinogen	Anti-human B_1C	Antistreptococcal antigen	
								MA_{12}	$T_{12}M$
S. M. L.	19	8th month	Pre-eclampsia	1st	++	+			
C. R.	37	8th month	Pre-eclampsia	3rd	++	+			
C. V.	27	8th month	Pre-eclampsia	2nd	++	++			
F. M.	42	9th month	Pre-eclampsia	13th	+	++			
C. A.	23	9th month	Pre-eclampsia	1st	++	+			
P. D.	38	8th month	Eclampsia	1st	++	+	−	−	−
A. G.	27	9th month	Eclampsia	1st	++	+++			
P. M.	24	9th month	Eclampsia	1st	++	+++			
O. C.	29	Puerperium (1st hrs.)	Eclampsia	1st	+	+++			
D. B. R.	21	Puerperium (3rd day)	Eclampsia	1st	+	++++			
D. A.	29	Puerperium (15th day)	Eclampsia	1st	+	++++			
T. R.	28	Postpartum (8 months)	Pre-eclampsia	3rd	−	−			
B. G.	29	Postpartum (2 years)	Pre-eclampsia	1st	−	−			
G. M.	43	Postpartum (3 years)	Pre-eclampsia	2nd	−	−			
P. T.	24	Postpartum (3 years)	Pre-eclampsia	1st	−	−			
F. A.	39	Postpartum (3 years)	Pre-eclampsia	2nd	−	−			

swelling either decreases or completely disappears while thickening of the basement membrane and glomerular adhesions persist (Fig. 4); (3) In all the patients examined, glomerular lesions varying in amount and distribution were still observed months and even years after eclampsia. In several cases a slight increase in NPN and in blood pressure levels were found (Figs. 5, 6, 7); (4) No significant alterations of the stroma and the interstitial renal vessels were observed except for 3 cases shown respectively in the 2nd, 3rd and 4th year following delivery where both light (Fig. 8) and electron microscopy revealed swelling of the basement membrane and subendothelial proliferation of the walls of the interstitial arterioles; (5) With the immunofluorescent studies (table) in 11 cases

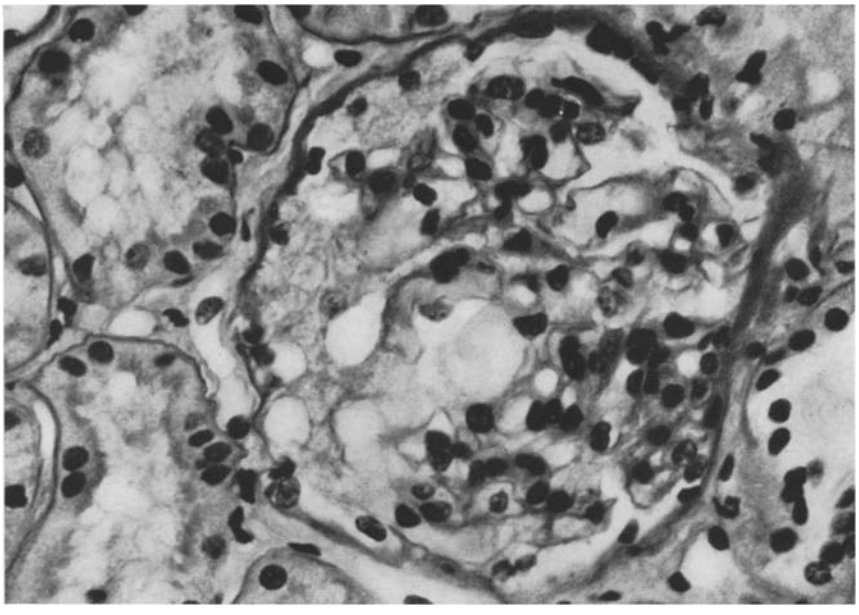

Fig. 1. Optical microscopy; Schiff reagent staining. Eclamptic 24 year old at first delivery; biopsy taken in the ninth month of pregnancy. Glomerular capillaries with obliterated lumen are seen. Basement membranes appear swollen and in places layered. PAS-positive material is observed in the lumen

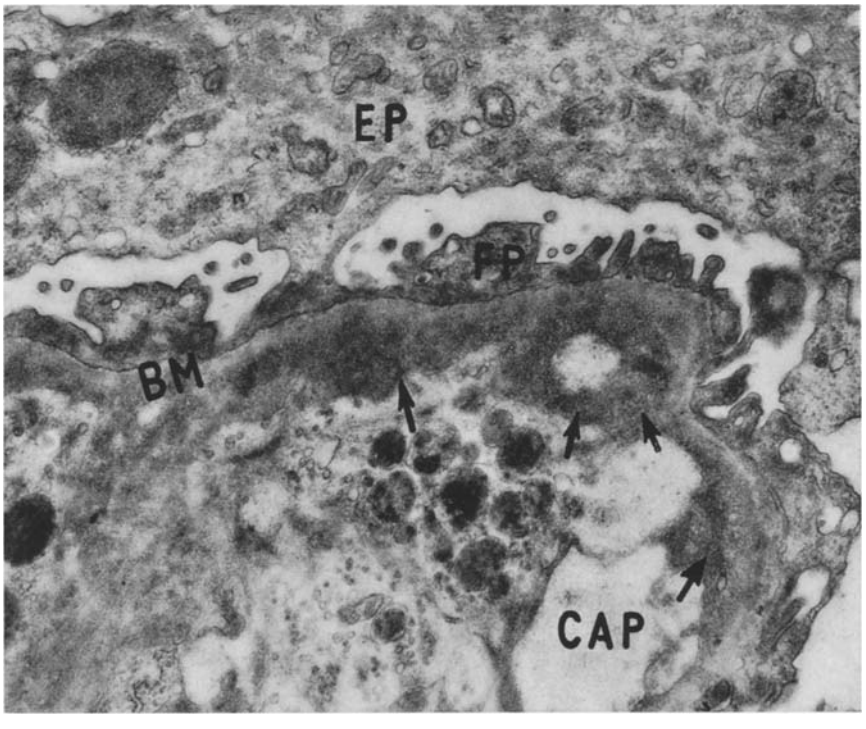

Fig. 2. Electron microscopy; Vestopal embedding; lead hydroxide staining. Eclamptic 25 year old at first delivery; biopsy taken at ninth month of pregnancy. The lumen of one glomerular capillary (*CAP*) appears completely filled with cellular debris. The basement membranes (*BM*) are irregularly thickened. Clumps of electron-dense material are seen in the lumen and under the basement membranes (arrows). The foot processes are fused at various points (*FP*)

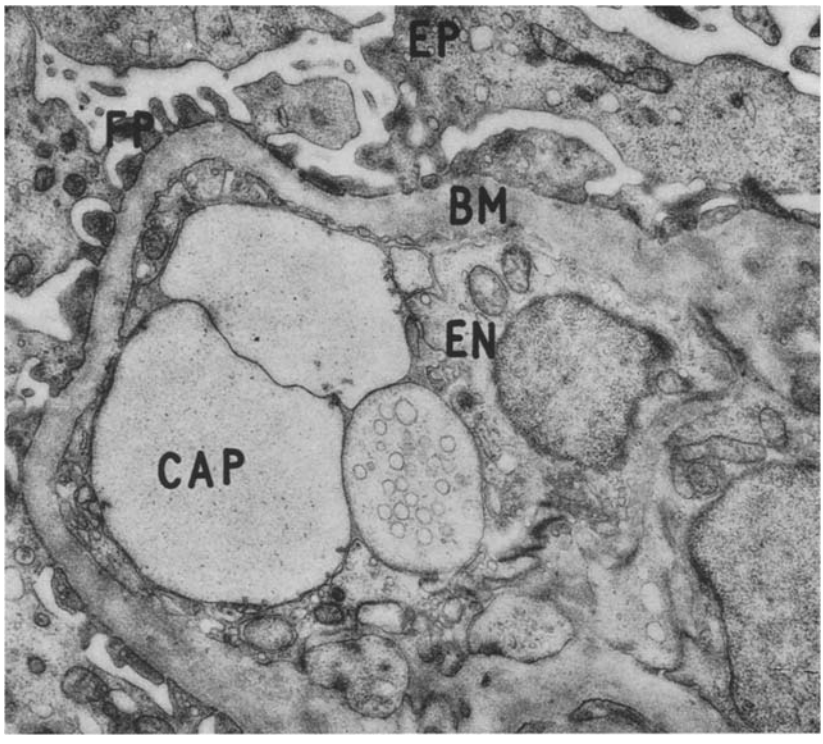

Fig. 3. Electron microscopy; Araldite embedding; lead hydroxide staining. Eclamptic 22 year old at first delivery; biopsy at the seventh month of pregnancy. A glomerular capillary (*CAP*) with its lumen almost completely occluded by swollen endothelium (*EN*) is seen. There is extensive thickening of the basement membrane (*BM*). The foot processes (*FP*) appear normal

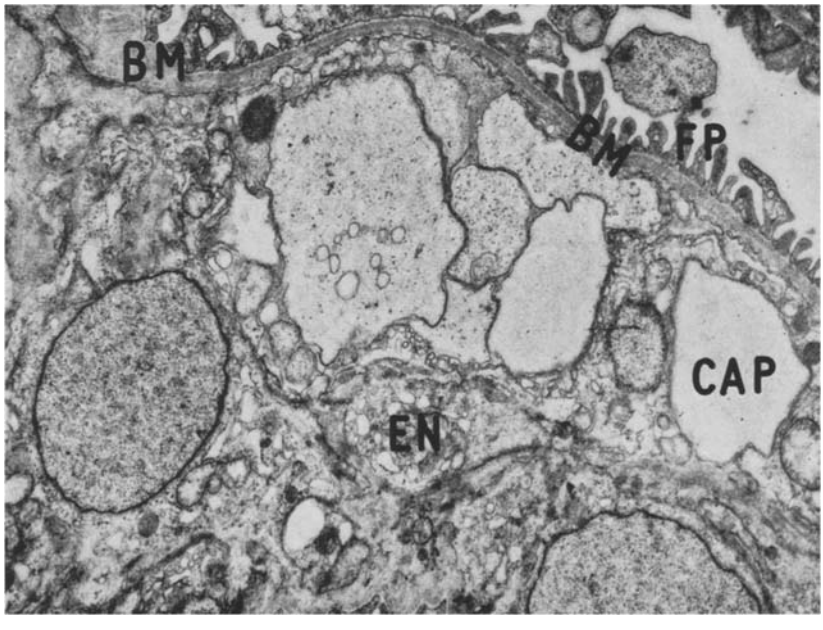

Fig. 4. Electron microscopy; Araldite embedding; lead hydroxide staining. Eclamptic 18 year old observed on the eighth day of the puerperium. The lumen on one capillary (*CAP*) is still partially occluded by the cytoplasm of an edematous and swollen endothelial cell (*EN*). The basement membrane (*BM*) is irregularly thickened. The foot processes appear normal

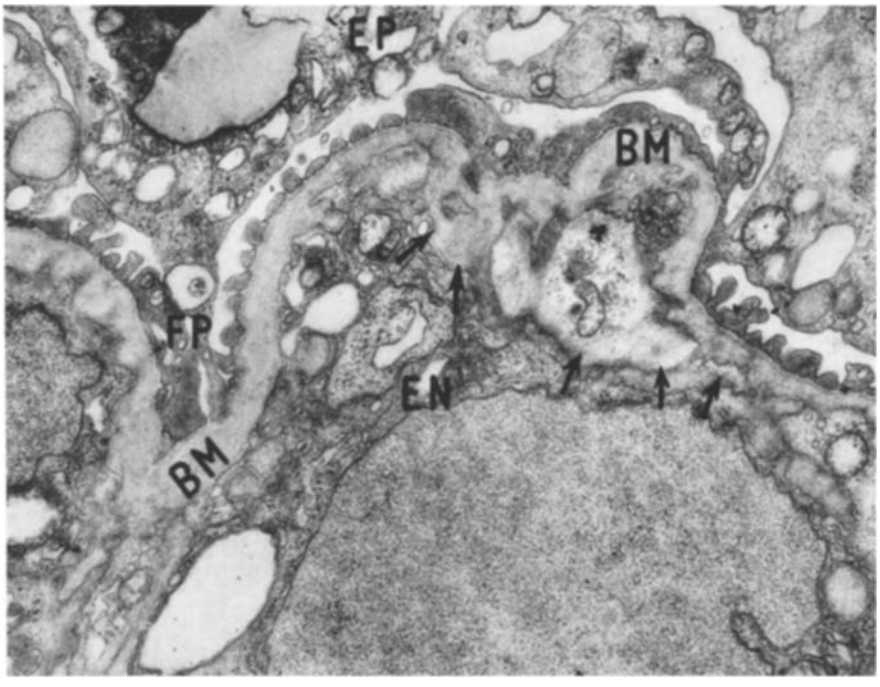

Fig. 5. Electron microscopy; Vestopal embedding; lead hydroxide staining. Twenty-six year old observed 2 years after her second delivery (B.P.: 120/60; NPN: 27 mg-%). The lumen of one capillary is completely obliterated by swollen endothelium (*EN*) and by clumps of electron-dense material (fibrils present in the central part). The basement membrane (*BM*) is thickened and, in general, the foot processes (*FP*) are normal

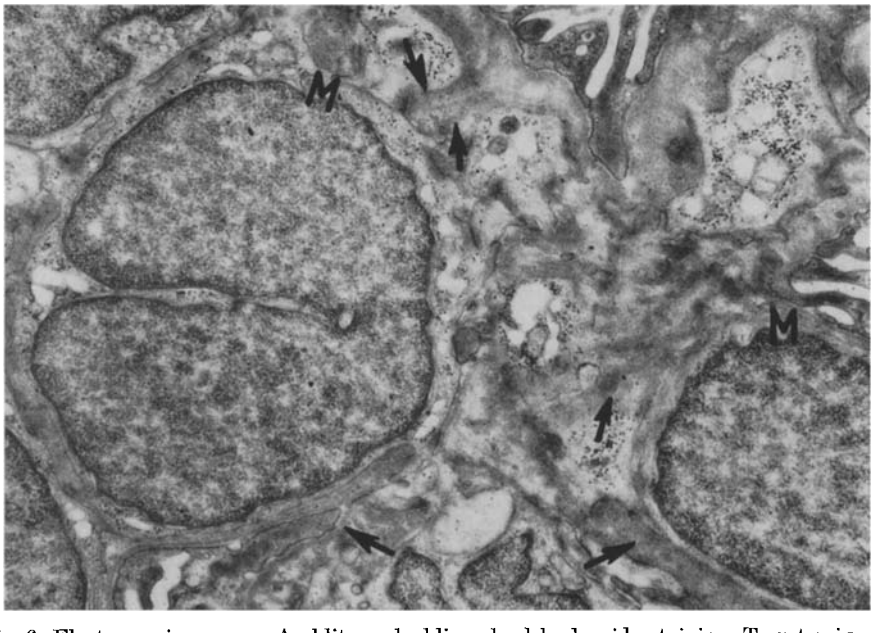

Fig. 6. Electron microscopy; Araldite embedding; lead hydroxide staining. Twenty-six year old observed 3 years after her first delivery (B.P.:120/80; NPN: 31 mg-%). Proliferation in the mesangial cells (*M*) with an increase of basement membrane-like material (arrows)

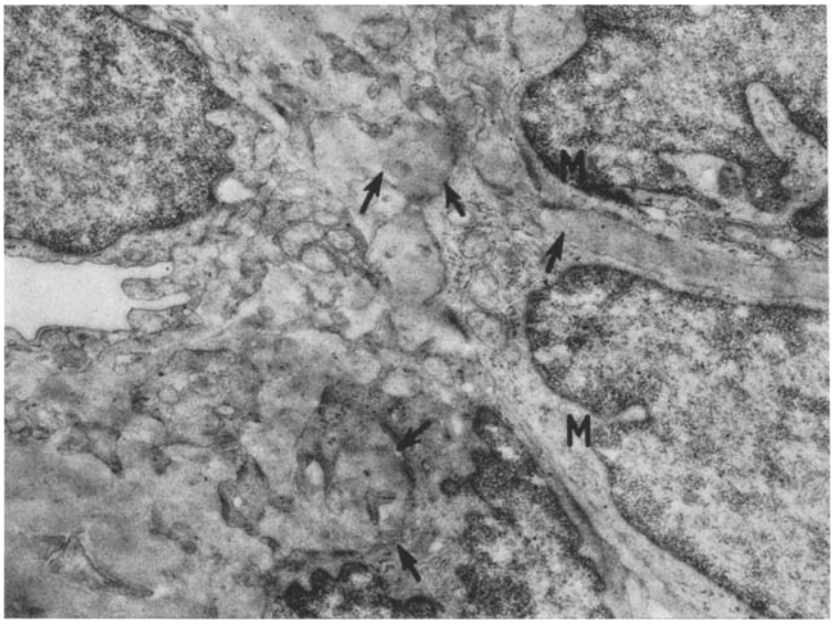

Fig. 7. Electron microscopy; Araldite embedding; lead hydroxide staining. Twenty-eight year old observed 4 years after her first delivery (B.P.: 130/80; NPN: 40 mg-%). Proliferation of the mesangial cells (*M*) with an increase of basement membrane-like material (arrows)

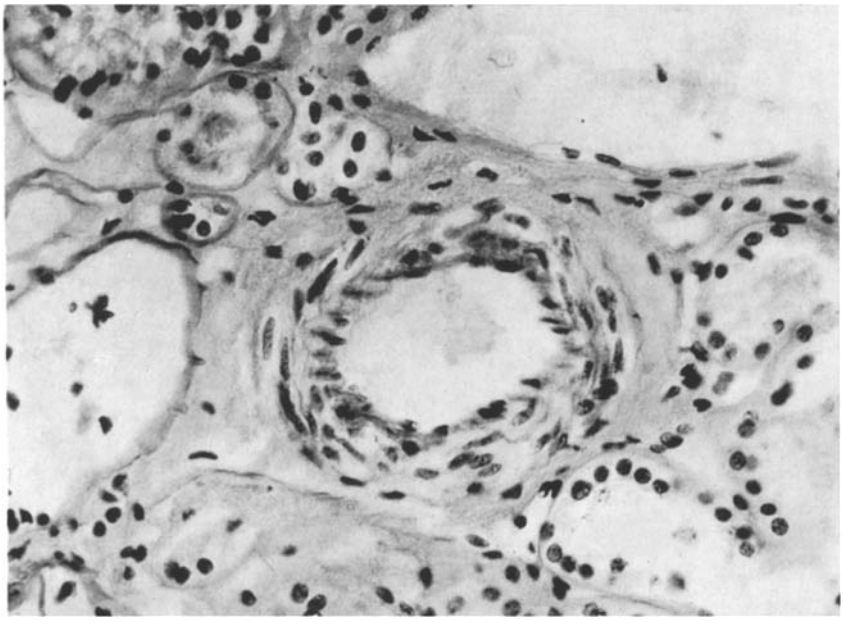

Fig. 8. Optical microscopy; Schiff reagent staining. Twenty-two year old observed 4 years after first delivery (B.P.: 100/60; NPN: 29 mg-%). The walls of an interstitial vessel are thickened with subendothelial proliferation

studied during pregnancy and in the puerperium, positive results were found for both the antihuman 7 S globulin (Fig. 9) and the antihuman fibrinogen (Fig. 10)

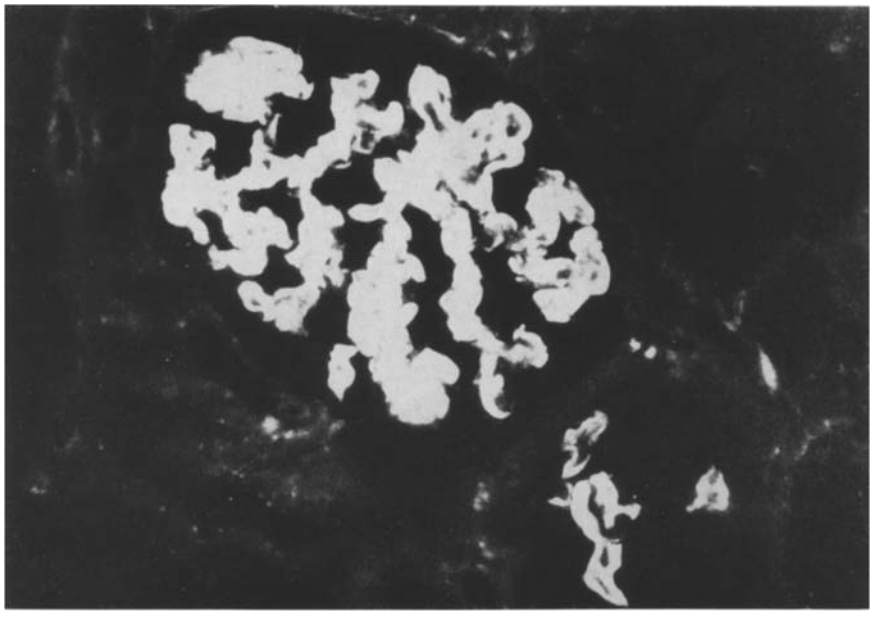

Fig. 9. Microscopy and fluorescence; cryostat preparation of frozen kidney section stored in dry ice until use. Renal glomerulus twenty-four year old woman at first delivery, affected with eclampsia at 9th month of pregnancy. Diffuse localization of gamma-globulin. Fluoresceinated antihuman 7S globulin

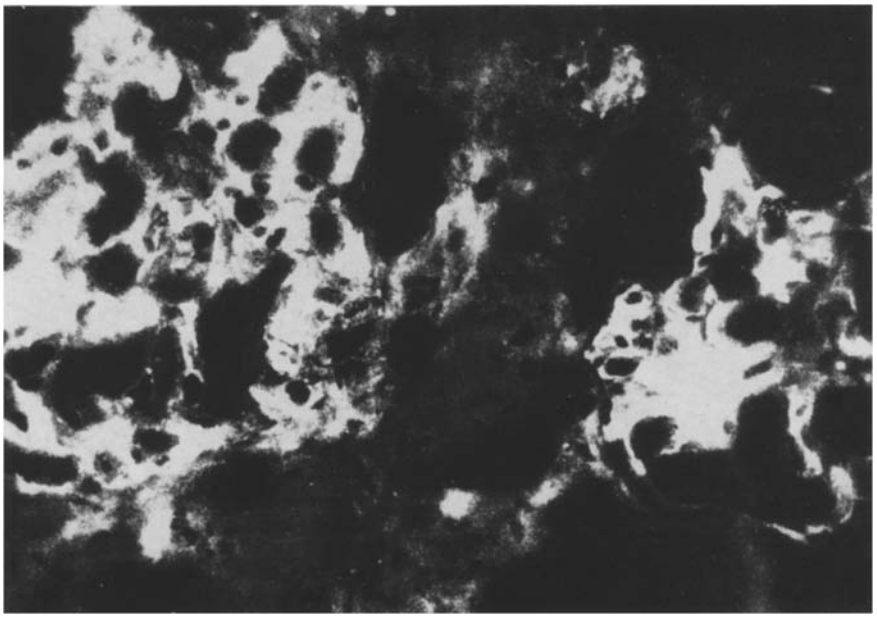

Fig. 10. Microscopy and fluorescence; cryostat preparation of frozen kidney section stored in dry ice until use. Renal glomerulus 24 year old woman at first delivery, affected with eclampsia at 9th month of pregnancy. Diffuse localization of fibrinogen. Fluoresceinated antihuman fibrinogen serum

sera. In the 5 patients observed in the first 3 years postpartum all the immunofluorescent reactions were negative. A negative result was obtained in the case tested with the antihuman B_1C complement and the anti-streptococcal antigen (MA_{12} and $T_{12}M$).

Discussion

In almost complete agreement with the findings cited in the literature (Cassano, 1960; Altchek, 1961; Hopper, 1961; Ishikawa, 1961; Fiaschi, 1962, 1962; Pontonnier, 1962), we could not find any significant difference between renal damage observed in eclampsia from that observed in pre-eclampsia. We have, however, confirmed that the lesions characteristically affect all the glomeruli although to a varying extent in any one biopsy.

We have studied kidney sections from 12 women taken during the first few days of the puerperium. Seven of these patients had had biopsies prior to delivery. Previous similar studies provide contradictory results, while some authors (Coppitz, 1957; Pollak, 1960; McCartney, 1964) maintain that the renal lesions completely regress in the first few hours after delivery, others (Hopper, 1961; Mautner, 1962; Dennis, 1963) have revealed that renal damage, although to a lesser degree, can still be present days and even months later. Our observations were made one hour to 30 days following delivery. Clinically we found that five patients continued to be hypertensive and one azotemic and twelve showed evidence of proteinuria. Our histological findings show that renal damage in toxaemia may not completely regress after delivery. Whereas the cellular edema was reduced, although not uniformly in all the glomeruli, the capillary loops occluded by endothelial proliferation and PAS-positive thickening of the basement membrane and the increased number of mesangial cells with an accumulation of electron-dense material in their cytoplasm persisted. In one woman examined 30 days following delivery, we found thickening of an interstitial arteriolar wall.

In the follow-up biopsies on the patients who had previously suffered from eclampsia or pre-eclampsia we had 17 specimens ranging from the first months to the fourth year postpartum. All the subjects appeared to be in good health at the time of biopsy and had not suffered from any significant intercurrent diseases. In three women we found slight increases in the pressure and/or NPN values. Morphological findings show structural alterations of varying degree involving the glomerulus in particular in all our patients. In those cases studied during the first year edematous and exudative alterations of the endothelium in numerous glomeruli, characteristic of the acute phase of toxaemia, were still present even though to a lesser degree. In the cases examined 2, 3 and 4 years following toxaemia, we found lesions of varying size and extension only in some of the glomeruli (2—3 per section). These lesions were characterized by localized or uniform thickening of the basement membranes, fusion of the loops, adhesions between visceral and parietal epithelium, and obliteration of several capillary lumens. In the 3 azotaemic subjects we found thickening of the subendothelial basement membrane with proliferation of endothelial cells of several arterioles of the interstitium. Endothelial edema and proliferation were most prominent immediately following the toxaemic episode. As the edematous portion of the endothelium regresses, alterations of the mesangium with increased production of basement membrane-like substance becomes more evident. This substance lines the inside

of the filtering membrane rendering it thicker then normal. Adhesions between the loops and Bowman's capsule form, thereby reducing the filtration space. The connective tissue-vascular axis of the glomerulus is retracted so that the glomerulus tends to be partially or totally sclerotic.

These observations have not previously been reported. Only Hopper (1961), observed in 3 subjects 5 weeks postpartum and Mautner (1962) in 2 women studied 5 months and 2 years postpartum have found modest and localized swelling of the endothelial cells and an increase in the intercapillary spaces and in the cytoplasm of the mesangial cells. In the light of these and our own findings we feel that the renal alterations in toxaemia in the eclamptic as well as the pre-eclamptic variety may not regress completely. Indeed, our studies concerning 29 specimens carried out in the first days and in the 1st, 2nd, 3rd and 4th year postpartum have consistently revealed the presence of glomerular lesions both by optical and electron microscopy.

The immunofluorescent studies have clearly shown that in the acute phases of the disease (table) one obtains positive reactions in the glomerular basement membrane with antihuman 7S globulin serum as well as with antihuman fibrinogen antibodies. Our relatively small number of patients does not permit us to draw definite conclusions as to the significance of these results.

Recently, Vassalli (1963), having found practically analogous results, suggested that positive reactions to antihuman fibrinogen serum favours an intravascular coagulation disturbance rather than a primary immunological one. The problem however is still undecided.

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Prof. E. Fiaschi
Istituto di Patologia speciale medica
Policlinico Universitario
Padova (Italia) 35100