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Immunofluorescent examination of the kidney post mortem*

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Summary. 106 selected kidneys removed at autopsy were studied by direct immunofluorescence using polyvalent antisera against human immunoglobulins, light chains, complement fractions and fibrinogen. The immunofluorescence was a suitable method to solve differential diagnostic problems that arose at autopsy. The diagnostic value was the most obvious in cases of immunologically mediated renal diseases and in immunologically mediated systemic diseases involving the kidneys. Negative immunofluorescence findings were also useful to determine the pathogenesis of renal lesions, especially in vasculopathies. The immunofluorescence of postmortem material showed similar disturbances to that obtained with biopsy material. At various sites, especially in the tubulo-interstitium, additional electron microscopical study was sometimes needed to localise the immune deposits exactly. The fluorescent microscopical examination of frozen sections of kidney taken at necropsy turned out to be more adequate than the immunoperoxidase examination of formalin-fixed, paraffin-embedded sections.

Key words: Immunofluorescence - Kidney - Autopsy

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Introduction

Immunohistochemistry is indispensable in the diagnostics of numerous diseases; in particular, as a consequence of its speed, simplicity, reliability and cheapness, the method of immunofluorescence (IF) has become a routine diagnostic procedure in pathology during the past 20 years. In addition to the light and electron microscopic examinations, it was initially applied as an integral part of the diagnostics relating to renal diseases. However, for a long time it was considered suitable only for the examination of fresh frozen material, i.e. samples obtained from living patients. During the past few years we have attempted to introduce this technique among our postmortem diagnostic procedures, and this paper presents an account of our results.

Materials and methods

From the routine autopsy material of the past 5 years, IF examinations of the kidney were performed in 106 cases. The indications were as follows: 1. Clinically postulated (confirmed by biopsy in 8 cases) renal diseases or systemic diseases affecting the kidney (81 cases). 2. The suspicion of a clinically undiagnosed renal disease or a systemic disease affecting the kidney, based on macroscopic observations (25 cases). Immunofluorescence can be utilized particularly well in the postmortem diagnosis of *lupus nephritis.* The frozen samples obtained in the course of autopsy were treated in a direct system with FITC-labelled anti-IgG, IgA, IgM, C_3, C_4, C_{1q} complement and fibrinogen DAKO or Behring sera in a dilution of 1:10. In some cases, anti kappa and lambda light chain sera were also used. In addition to light microscopy and IF, electron microscopy was performed in 7 cases and also in 7 other cases in which the PAP technique for the detection of the above mentioned substances was carried out in formalin-fixed tissues. The length of the period between death and autopsy varied between 3 and 72 hours.

Results

Table 1 lists those diseases where the IF gave positivity in the glomeruli, and/or in the vessels or the tubular basement membrane. Besides the tabulated data, certain additional observations are mentioned below. In 4 cases, on the basis of the continous linear positivity, the rapidly progressive glomerulonephritis (Fig. 1) proved to be an anti basement membrane antibody disease and was part of Goodpasture's syndrome, while in one case it was associated with hypersensitivity angitis. In this latter case, the positivity (fibrinogen) was only in the crescents and vessels. In 2 cases, the amyloidosis (Fig.2) accompanied myeloma multiplex, while the third case was AA type. The kappa light chain disease (Fig. 3) similarly occurred in a patient with myeloma multiplex; in this case, beside the negative glomerular finding, the diagnosis was based on the positivity of the tubular basement membrane. In malignant hypertension and in hypersensitivity angitis, the predominant sign was generally that the renal vessels were affected (Fig.4), but in one case in each group glomerular immune deposits were also observed. In addition to very high glomerular positivity (usually including C_{1q} complement), tubulo-interstitial fluorescence was often seen in lupus nephritis (Fig. 5). Postmortem IF was carried out in 8 cases where biopsy had previously been perfomed, but only 2 of these biopsy specimens were subjected to IF. In a case of mesangioproliferative glomerulonephritis, a period of 4 years had elapsed between the biopsy and death; the postmortem IF finding was substantially poorer than the biopsy result, corresponding to glomerulonephritis that had passed into the sclerotic phase. In the other case, the time between the biopsy and death was only 4 months, and here the postomortem IF agreed with that of the biopsy. In one case, where IF had not been performed on the biopsy specimen, IgA nephritis was discovered only by IF of the autopsy material (Fig.6).

In 63 cases the IF finding was negative; 5 of these cases represented chronic pyelonephritis and another 5 were end-stage kidneys (type unknown). In the other cases (polyarteriitis nodosa, rheumatoid arthritis, benign hypertension, Moschcowitz syndrome, minimal change glomerulonephritis, chronic pyelonephritis), positivity was observed at most with anti-fibrinogen serum. In myeloma multiplex (12 additional cases to the positive ones mentioned above) and in Waldenström's macroglobulinaemia (2 cases), kappa-or lambda-positive cellular infiltration was found in the interstitium; there were negative glomeruli in some cases.

In 7 cases, in addition to IF, the PAP procedure was also carried out on formalin fixed material and the results were compared. It was found that the antigen loss ocurring during the embedding was generally so extensive that it led to a decrease in intensity or even to the disappearance of the originally weak positivity. As a consequence of the intense background staining that occurred, particularly with the anti-IgG serum (which could not be diminished appreciably with the methods recommended in the literature) (Bourne, 1983), assessment of the tubulo-interstitial immune deposits was considerably easier with IF.

IF of the autopsy kidneys proved to be of diagnostic value in cases of Goodpasture's syndrome. SLE, IgA nephritis, kappa light chain disease and minimal change glomerulonephritis.

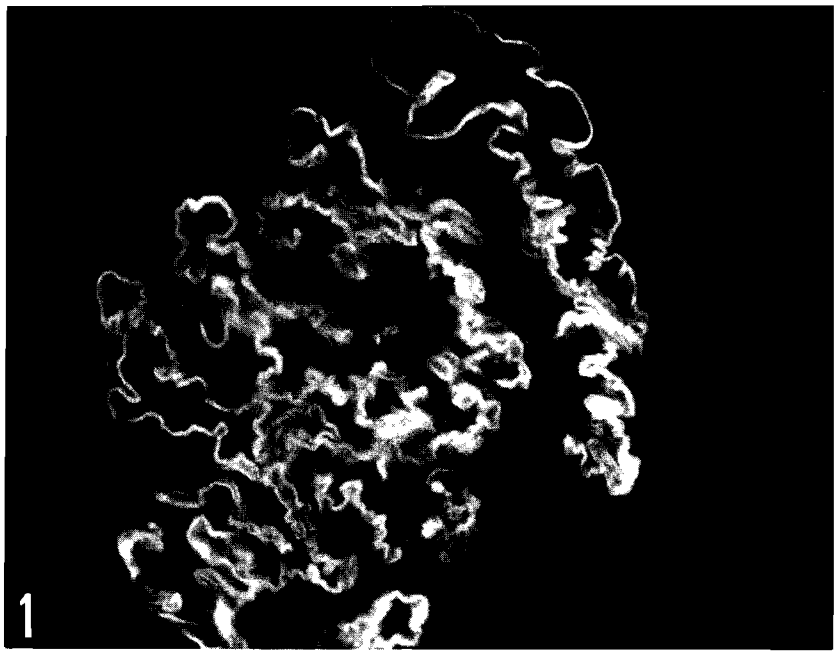


Fig. 1. Rapidly progressive glomerulonephritis. Anti basement membrane antibody disease. Linear positivity along the glomerular capillary loops. Anti-kappa serum x160

Fig. 2. Amyloidosis associated to myeloma multiplex. IgG is situated in accordance with the amyloid. x400

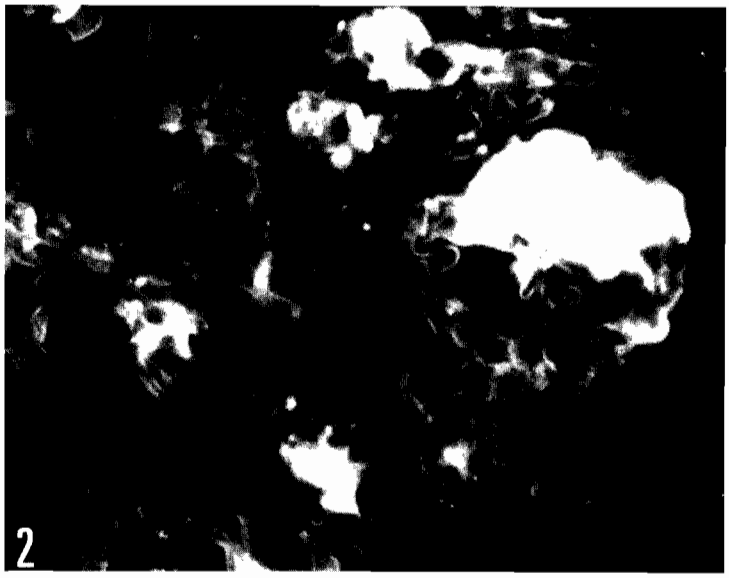


Fig. 3. Kappa light chain disease in a myeloma multiplex patient. Linear staining for kappa along the tubular basement membranes, with a double contour in places (arrow). x400

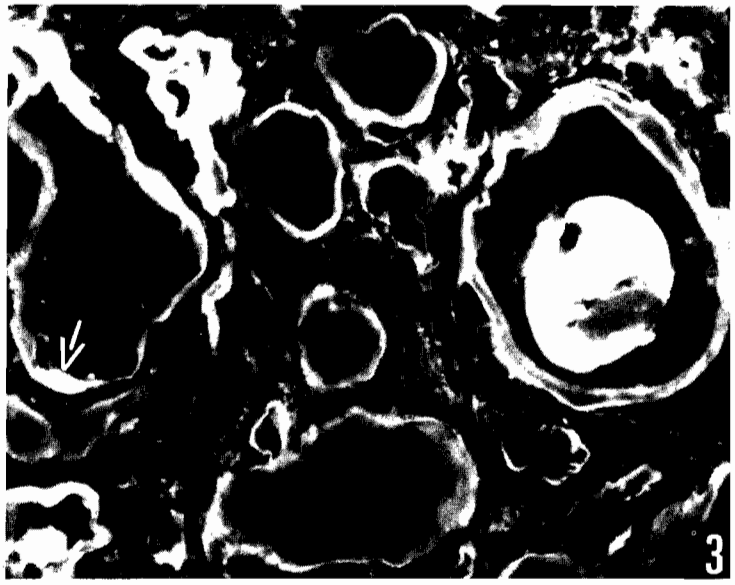
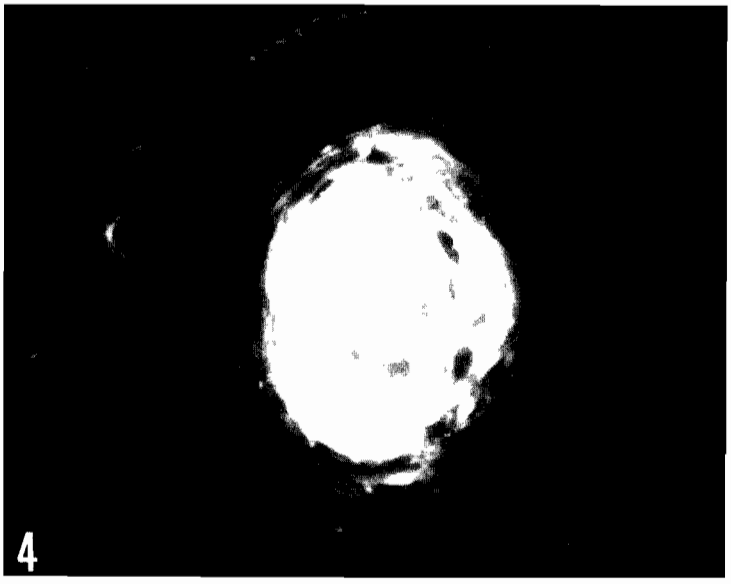


Fig. 4. Malignat hypertension. Fibrinogen permeating the intima and in part the media of a medium-sized artery. x400



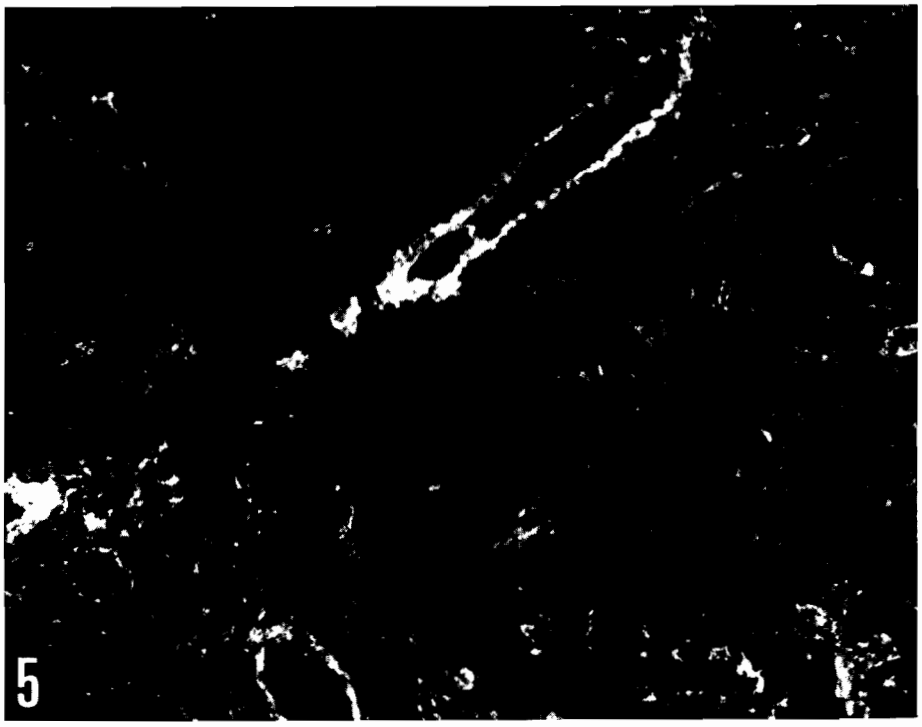


Fig. 5. Systemic lupus erythematodes. Tubulo-interstitial positivity. Anti-kappa serum. x160

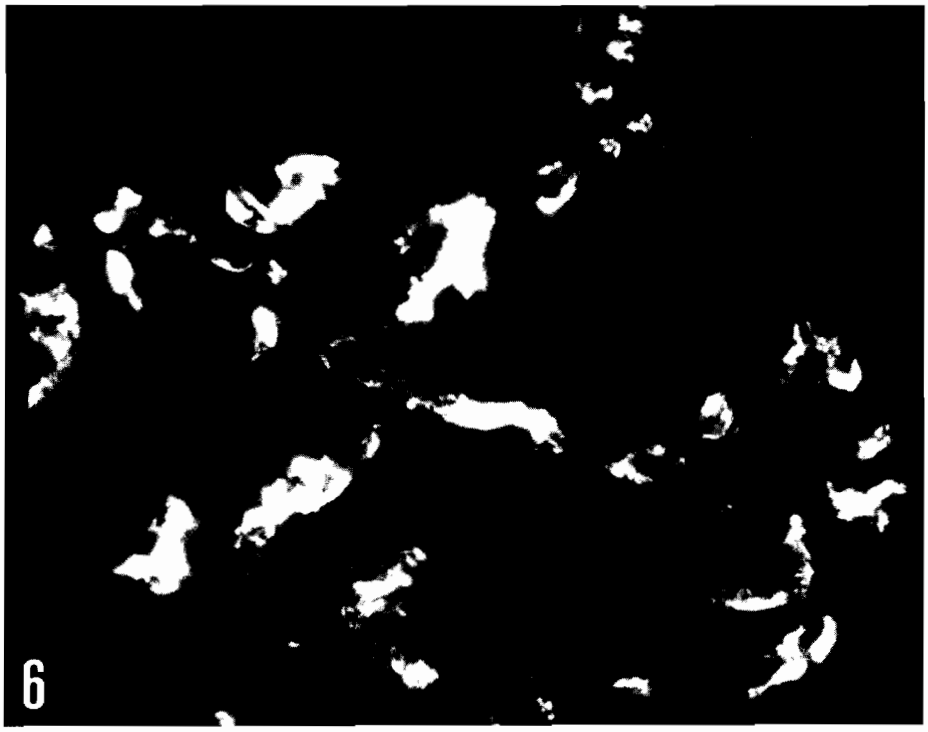


Fig. 6. IgA glomerulonephritis. Purely mesangial localisation of IgA. x400

Diagnosis	No. of Cases (n)	Localization of positivity	IgG	IgA	IgM	C3	C4	Clq	fibr.	kappa	lambda
Diff. scler. GN	8	Mesangial	2	3	2	2					
		Peripheral	2	3	2	3					
		Crescent							1		
		Vascular wall			1	1					
Mesangiocap. GN I.	2	Mesangial		1	1	1					
		Peripheral	1	1	1	2					
		Bowman-capsule				1					
		Vascular wall	1		1						
Dense deposit GN	1	Mesangial	1			1					
		Peripheral	1			1					
		Vascular wall	1								
IgA GN	2	Mesangial	2	2							
		Peripheral	1	1							
Crescentic GN	5	Linear glom. BM	4		2	4				1	1
		Crescent							4		
		Bowman-capsule	1							1	1
		Linear tub. BM	1						1	1	
		Tubulointerst. cell	2	2	2					2	2
		Vascular wall						1	1		
SLE	10	Mesangial	7	3	4	3		5		2	2
		Peripheral	8	6	5	6		7	1	3	3
		Focal segmental							1		
		Crescent							2		
		Tubulointerst.	4	2	1	2		5		3	3
		Vascular wall	5	2	2	1		5	3	1	1
Amyloidosis	3	Diff. glom.	2	1	2				1		1
		Mesangial				1	1	1			
		Focal segmental	1						1	1	1
		Vascular wall						1			
Kappa light chain disease	1	Linear tub. BM								1	
		Tubulointerst.								1	
Scleroderma	2	Focal segmental	1								
		Vascular wall	2		1			1			
Malignant hypertension	6	Peripheral	1	1		1					
		Bowman-capsule							1		
		Vascular wall	1		1			1	1		
Hypersensitivity angitis	3	Peripheral							1		
		Bowman-capsule							1		
		Crescent							1		
		Inflamm. cell	1	1	1					1	1
		Vascular wall	1								

Abbreviations: Ig = immunoglobulin; C = complement; fibr. = fibrinogen; Diff. = Diffuse; scler. = sclerosing; GN = glomerulonephritis; mesangiocap. = mesangiocapillary; SLE = Systemic lupus erythematodes; glom. = glomerular; tub. = tubular; BM = basement membrane; Inflamm. = Inflammatory

Discussion

As occurs with biopsy specimens,postmortem IF is extremely important in the diagnosis of renal diseases with various aetiopathogenesis. A good result could be attained virtually without exception if the autopsy material examined was no older than 24 hours, but sometimes evaluable results were also observed even after 72 hours. Its speed, and the possibility of demonstrating the presence or absence of immune deposits, as also their nature and location, made it an ideal method for the clarification of differential diagnostic problems that arose at autopsy. In contrast with the PAP technique on formalin-fixed material, the assessment of positivity was usually much easier, particularly with regard to tubulo-interstitial deposits; IF is therefore preferable to the PAP technique. A negative finding in numerous diseases (e.g. minimal change and various vasculopathies) is at least as informative as the positivity otherwise. Our results, which were obtained almost exclusively by IF examination of biopsy material conform with the characteristic forms of the disease (Beregi and Varga, 1978; Meadows, 1978; Zollinger and Mihatsch, 1978; Valenzuela and Deodhar, 1980; Churg and Sobin, 1982; Heptinstall, 1983; Bohle et al., 1984). IF examination of renal tissue obtained at autopsy has been dealt with by Scheibani et al. (1979). Their results do not differ from our own. IF examination of postmortem material may provide similar, but more useful, data to that obtained from biopsy material. Biopsy material can also generally be examined electron microscopically which, in part, may be a substitute for the IF examination; postmortem material is on the other hand much less suitable for this purpose. It is true that certain ultrastructural glomerular changes may also be recognized in postmortem material (Johannessen et al., 1979); nevertheless, electron microscopic examination which is far more expensive and time-consuming is also disturbed much more than the IF examination by postmortem damage. After death, the material becomes more quickly unsuitable for electron microscopic processing than for IF examination. When the autolysis is so advanced that IF examination becomes hopeless, even customary histological processing permits, at best, only the demonstration of certain major changes.

The usefulness of the IF and peroxidase (PAP) techniques in renal biopsies was reported by Kemény et al. (1983), who listed the advantages of the latter method: retrospective processing, comparison with other staining procedures, and a lasting specimen. These also hold for kidney tissue obtained at autopsy. However, an IF examination of the kidney carried out directly at the autopsy can not be fully replaced by the PAP method, which is always performed later.

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