reducing or preventing subsequent staining of the sections with fluorescein-conjugated anti-DPO serum. Accordingly, the sections were washed six times with BPO-propylamine or with DPO-amylamine 2×10^{-2}M, washed thoroughly with saline solution and then stained with the fluorescein-conjugated anti-DPO antiserum. No reduction in staining with the anti-DPO serum was seen. On the contrary, staining of glomerular and tubular basement membranes appeared to be enhanced. The staining in areas of tubular necrosis was markedly enhanced; in these areas, clusters of brightly stained droplets appeared that were not evident in sections that were not first washed with hapten. These findings indicate that the DPO hapten detected was bound to kidney structural proteins rather than being part of a deposited antigen-antibody complex. The enhancement of staining produced by washing with hapten may have been due to specific elution of antibody, which would make more DPO haptenic sites available to bind the fluorescein-conjugated anti-DPO antiserum. This enhancement did not appear to be due to binding of DPO-amylamine by the tissue, since treatment of sections from other kidney diseases in the same fashion did not cause staining with the anti-DPO serum.

Sections were also stained for IgM, IgG, IgA, fibrinogen and complement ($beta_1A$-$beta_1C$). IgC was seen in a pattern similar to that observed for DPO hapten, whereas no staining was seen for IgA, IgM, complement or fibrinogen. Gamma-globulin staining appeared to be specific, since the antiserum did not stain normal kidney, and specific staining was obliterated by absorption of the antiserum with purified gamma globulin. Whether or not the gamma globulin was antipenicilloyl antibody could not be determined.

Discussion

The sequence of events in the seven cases included in the present report leaves little doubt that in each the nephropathy could be attributed either to methicillin or to penicillin therapy. The clinical picture was remarkably similar in the seven patients, and in all, high doses of the drug were used for relatively long periods. The histologic findings were principally interstitial nephritis and tubular damage. Arteritis or glomerular lesions were not seen by light microscopy. Similar pathological findings have been described as manifestations of methicillin reactions in two reports (16, 17) and of a penicillin reaction in one patient who had also received, sulfonamide and died with sepsis. (2) In addition, several cases of interstitial nephritis due to drugs have been reported in association with phenindione (26, 27) and sulfonamide therapy. (28, 29) Although we and others have referred to the lesion as interstitial nephritis, tubular damage was present as well, and it is not clear whether the initial event occurs in the tubules, the tubular basement membrane or the renal interstitium.

The pathogenesis of the nephropathy that occurs with methicillin or penicillin is unknown, but several considerations indicate that the lesions result from hypersensitivity. In the first place, in our patients a syndrome characterized by fever, rash and eosinophilia, which is generally accepted as evidence of an allergic reaction, developed. Secondly, only a small number of patients receiving methicillin or penicillin, even in very large amounts, have such a reaction, suggesting that it is not due to direct toxicity of the drug. Thirdly, DPO hapten (derived from methicillin) and gamma globulin were found in the kidney of the one patient studied by immunofluorescence (L.C.). Fourthly, the same patient, whose antibody response to penicillin was investigated, was found to have an unusually intense immune response, characterized by a high IgM titer, the presence of IgG and skin-sensitizing antibodies against penicilloyl determinants, as well as by delayed hypersensitivity. This kind of immune response is rarely observed in patients treated with penicillin without an allergic reaction. (24, 30) The IgG, IgM and skin-sensitizing antibody were shown to be specific for the benzylpenicilloyl group rather than the dimethoxyphenylpenicilloyl group. (31) The observation that serum antibodies specific for the benzylpenicilloyl group rather than for the dimethoxyphenylpenicilloyl group were stimulated by methicillin may be due to an anamnestic immune response, because he had been treated with benzylpenicillin three years previously. (31) The delayed hypersensitivity, however, appeared to be specific for methicillin rather than for penicillin, as indicated by the results of delayed skin tests to these drugs. Delayed hypersensitivity may thus represent a primary immune response.

These considerations favor a hypersensitivity basis for the renal lesions. How-