should be noted that the earlier measurements were made in an area away from the trees.

This investigation points up the desirable features of thermoluminescent microdosimetry for ecological radiation studies. These are: small size, low cost, ruggedness and ease of handling, reproducibility, and high sensitivity and working range, as well as long-term stability. A reasonable conclusion from this experiment is that a large increase in environmental dose rate occurs at the outset of spring growth in a forest environment with the development of foliage.

WILLIAM C. ASHBY*
JAMES N. BEGGS†, JACOB KASTNER
B. G. OLTMAN, HARRY MOSES
Radiological Physics Division,
Argonne National Laboratory,
Argonne, Illinois 60439

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11. Work performed under the auspices of the Atomic Energy Commission.
* PACE resident associate from Southern Illinois University, Carbondale.
† Argonne semester student from St. Olaf College, Northfield, Minn.

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Autologous Immune-Complex Pathogenesis of Experimental Allergic Glomerulonephritis

Abstract. *A renal tubular epithelial antigen is deposited in association with gamma globulin and complement in glomeruli from rats with experimental allergic glomerulonephritis induced by immunization with renal tubular antigens. Apparently, in normal kidneys this antigen is concentrated in the distal segment of the proximal convoluted tubular epithelium, and the principal source of this antigen in the glomerular deposits is autologous. This form of glomerulonephritis provides an experimental prototype for what may be termed "autologous immune-complex" diseases.*

Immunologically induced experimental glomerulonephritides may be pathogenetically divided into two categories. Some are the result of a direct and immunologically specific interaction of antibody with glomerular antigens, whether mediated by heterologous antibody as in nephrotoxic nephritis (*1*), or by autologous antibody as observed in sheep and rabbits after immunization with glomerular basement membrane (*2*). These nephritides are characterized by linear deposits of γ-globulin and complement components along the endothelial margins of glomerular basement membranes (*3*). Others are those forms of glomerulonephritis which are the consequence of the deposition of circulating antigen-antibody complexes immunologically unrelated to the glomeruli (*4*). These are characterized ultrastructurally by electron-dense inhomogeneous deposits along the epithelial aspects of the glomerular basement membranes as well as a granular distribution of γ-globulin and complement along the basement membranes of diseased glomeruli (*5*). In the immune-complex forms of disease the antigen may be of exogenous origin (the foreign proteins in serum sickness) or autologous as proposed for the nuclear antigens in lupus erythematosus (*6*).

Among immunologically mediated experimental renal diseases, one model has a remarkable degree of similarity to certain forms of the nephrotic syndrome in man (*7*). This disease, experimental allergic glomerulonephritis induced in rats by immunization with tubular antigens (TA), is a chronic membranous glomerulonephritis manifested clinically by proteinuria, hyperlipemia, and hypoalbuminemia characteristic of the nephrotic syndrome (*8*). As in other forms of nephritis mediated by immune complexes, γ- and β_{1C}-globulins of the host are present as granules distributed along the glomerular basement membrane (*9, 10*), and inhomogeneous lumpy deposits of electron-dense material are found along the epithelial aspect of the glomerular basement membrane by electron microscopy (*11*), thus fulfilling the currently established immunofluorescent and morphologic criteria for an immune-complex pathogenesis.

A distinct relation between dose of immunizing antigen and severity of experimental allergic glomerulonephritis has been noted (*12*), and in the conventional program of induction, quite sizable amounts of antigen (2 to 5 mg) in complete adjuvant are given intraperitoneally to the rats weekly or semiweekly. This has raised the possibility that the disease might represent nothing more than a variation on chronic serum sickness in which administered foreign protein is the sole or major antigenic participant in formation of circulating antigen-antibody complexes with secondary deposition in the renal glomerulus (*10*). However, if autologous antigen participates in formation of antigen-antibody complexes, then this form of experimental allergic glomerulonephritis is an example of an "autologous immune-complex" disease.

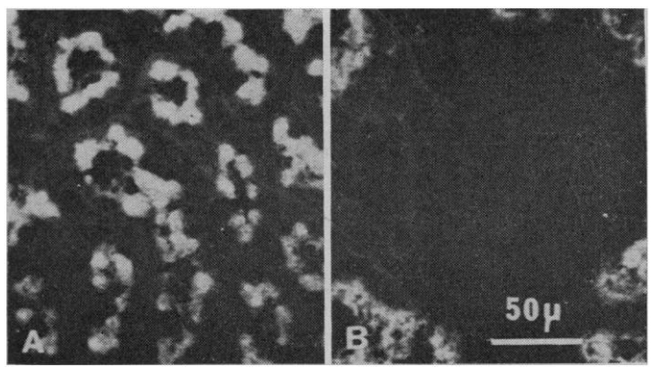
To test this hypothesis, we used immunofluorescent methods. Antiserums to renal tubular epithelium were produced by repeatedly injecting rabbits with the ultracentrifugal sediment (78,680*g*) of a supernatant (400*g*) of sieved homogenate of rat kidney emulsified in incomplete adjuvant. This tissue fraction is referred to as fraction 1A (Fx1A) (*13, 14*). After absorption with lyophilized rat serum, liver, and supernatant (78,680*g*) of rat kidney homogenate, all of which are devoid of the specific nephritogenic antigen (*14*), the γ_2-globulin fractions of such absorbed rabbit antiserums (antibody to rat TA) were used in the indirect immunofluorescent technique (*15, 16*). In the normal rat kidney, staining was particularly strong in the inner cortical zone where the antibody to rat TA localized in the brush border and apex of the tubular epithelium (Fig. 1) which, on the basis of histologic features and location in the kidney, represents the distal portion of the proximal convoluted tubules (*17*). The antibody to rat TA reacted with neither normal glomeruli nor glomeruli in kidney sections from rats with nephrotoxic ne-

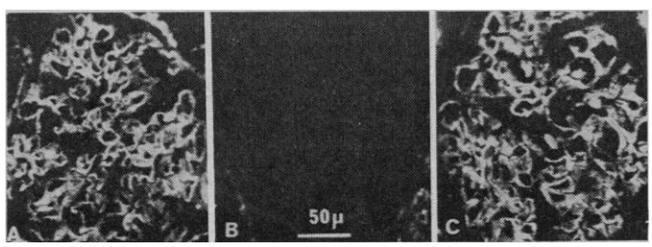
phritis or aminonucleoside nephrosis.

Although granular glomerular deposits of γ-globulin and complement had been demonstrated in allergic glomerulonephritis induced by TA (*9, 10*), the antigenic constituent of the deposited immune complexes had not been identified. To evaluate the presence of a tubular antigen in the glomerular-deposited immune complexes, kidney sections from rats with severe disease (*12*) were exposed to antibody to rat TA in the indirect immunofluorescent method. There was unequivocal but weak granular staining along the glomerular basement membranes, which was weaker but otherwise indistinguishable in character from that observed for γ-globulin and β_{1C}-globulin. To increase staining of the tubular antigen in the glomerular deposits, partial dissociation of the immune complexes was attempted. Acid elution of sections from the diseased kidneys usually resulted in a moderate loss of all of the immune-complex constituents; therefore 2.5*M* potassium thiocyanate (KSCN) was explored as a dissociating agent. Dandliker *et al.* suggest that dissociation of antigen-antibody complexes is due largely to the rupture of hydrophobic bonds (*18*). After elution of normal kidney sections with 2.5*M* KSCN, only the usual tubular epithelial staining was found. However, the typical granular staining of glomerular-deposited TA in diseased kidney sections was distinctly augmented. Staining of the glomerular-deposited tubular antigen was blocked by absorption of antibody to rat TA with rat Fx1A but not by absorption with whole rat glomeruli or glomerular basement membrane (*13*), nor by absorption with rat lung, heart, small bowel, stomach, brain, prostate, seminal vesicle, skeletal muscle, spleen, testis, or whole blood, thus indicating the renal tubular epithelial specificity of this antigen. Elution of unfixed cryostat sections in 2.5*M* KSCN for 30 minutes to 2 hours at 37°C and 15 minutes at 56°C was best.

The sections were then washed in saline, fixed, and stained (*16*). Evidence for partial dissociation of the glomerular-deposited immune complexes is offered by a consistently observed decrease in staining for γ- and β_{1C}-globulins following KSCN elution, which was apparently due to diffusion of these dissociated constituents from the immune complexes in a manner analogous to that observed for immune complexes in vitro (*18*) or in the recovery of antibodies to nuclear material by acid extraction of the glomeruli of kidneys from patients with lupus erythematosus (*19*). In contrast, the antigen appears to remain at the site of the complexes, possibly due to its large size and other physicochemical properties (*20*).

To determine whether or not the tubular antigen deposited in diseased glomeruli was of autologous origin or was derived from the immunizing antigenic pool, the disease was induced with rat Fx1A in one group of rats and with human Fx1A in a second group (*8, 12*), and the species origin of the glomerular-deposited antigen in each was determined in KSCN-eluted kidney sections with appropriate antiserums. Rabbit antibody to human TA was prepared, with human kidney fractions, by the method described for preparation of antibody to rat TA. After absorption of antibody to rat TA with human Fx1A and absorption of anti-





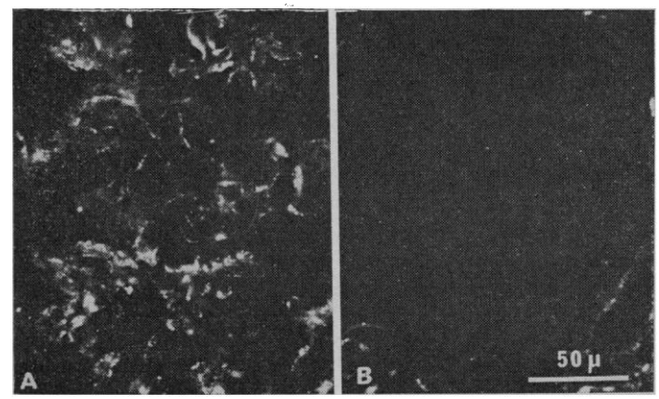


Fig. 1 (upper left). Specificity of antibody to rat TA, as shown by immunofluorescence. (A) Considerable specificity (particularly strong in the inner or juxtamedullary region of the normal rat kidney) for the brush border and apex of proximal convoluted tubular epithelium. (B) Section of normal kidney (eluted in 2.5*M* KSCN for 2 hours at 37°C and 15 minutes at 56°C) showing no glomerular staining. Uneluted normal kidney was similar. Antibody to rat TA apparently possesses immunohistochemical specificity for antigens of the proximal convoluted tubular epithelial cells of the normal renal cortex.

Fig. 2 (lower left). Glomerular-deposited antigen and the species specificity of the antigens in kidneys from rats with experimental allergic glomerulonephritis induced with human kidney Fx1A, as shown by immunofluorescence. (A) Presence of tubular epithelial antigen along the glomerular basement membrane shown with antibody to rat TA after elution of the section in 2.5*M* KSCN. The antigen is deposited in a very finely granular pattern which appears nearly membranous at lower magnification. (B) After absorption of antibody to rat TA with rat Fx1A, staining of the glomerular-deposited antigen is completely blocked in an adjacent section. (C) Absorption of antibody to rat-TA with human kidney Fx1A produces no discernible attenuation of the staining seen in (A). Most of the antibodies responsible for staining the glomerular-deposited antigen are apparently directed to antigenic groups species-specific for the rat; this indicates the autologous origin of the glomerular-deposited antigen. Fig. 3 (upper right). Traces of antigen with specific antibody to human-TA (absorbed with rat Fx1A), as shown by immunofluorescence. (A) Typical glomerulus from organ with disease induced with human Fx1A. At this magnification the fine granular character of the deposited human antigen is seen. (B) The same antiserums failed to stain glomerular-deposited antigen in experimental allergic glomerulonephritis induced with rat Fx1A.

Table 1. Species of origin of glomerular-deposited antigen in experimental allergic glomerulonephritis, as shown by immunofluorescence. Antibody was γ_2-globulin, first layer of indirect method of fluorescence. For absorption, 15 mg of antigen per milligram of antibody was used.

Absorbing agent	Disease-inducing antigen	
	Rat antigen	Human antigen
Antibody to rat TA		
None	4+	4+
Rat Fx1A	0	0
Human Fx1A	4+	*4+**
Antibody to human TA		
None	2+	2+
Rat Fx1A	0	*1+**
Human Fx1A	0	0

* The italicized results are most significant. Specific antibody to rat TA (absorbed with human Fx1A) gave *4+* staining of the glomerular-deposited antigen in disease induced with human antigen, thus demonstrating the presence of autologous (rat) antigen. Specific antibody to human TA (absorbed with rat FxIA) weakly stained (*1+*) the glomerular-deposited antigen, thereby establishing the presence also of a small amount of administered antigen.

body to human TA with rat Fx1A, the antiserums were species-specific.

The specific antibody to rat TA stained the glomerular-deposited antigen in a uniform and dense beading along the glomerular basement membranes (Fig. 2C), identical to the original antibody to rat TA (Fig. 2A) in disease induced with either antigen (Table 1). Thus, while human Fx1A was incapable of absorbing the responsible antibodies, absorption with rat Fx1A completely abolished the fluorescent antibody reaction (Fig. 2B). In a few cases of disease induced with human Fx1A, it was possible to omit the elution in KSCN and still identify autologous (rat) tubular antigen in the deposits. Autologous (rat) antigen must then have participated in immune complex formation when the disease was induced with human Fx1A.

When the kidneys from rats receiving human Fx1A were stained with specific antibody to human TA, a faint, irregular, and occasionally patchy but distinctly granular staining of glomerular deposited antigen was observed (Fig. 3), while no staining of glomeruli from rats receiving rat Fx1A was seen (Table 1). The quantitative relationships between the amounts of human and rat tubular antigen in the deposits of animals injected with human Fx1A are not established. However, the distinctly weaker and less regular staining of human antigen, regardless of the concentration of specific antibody to human TA, suggests that only a minor proportion of the glomerular-deposited antigen in these kidneys was derived directly from the immunizing material.

Although some of the immunizing antigen appears to participate in immune complex formation in circumstances where repeated large doses of antigen are employed (totaling approximately 50 mg), this is probably not a necessary feature of this disease. Typical experimental allergic glomerulonephritis may be induced by a single immunization, divided between the two rear footpads, with doses down to 500 μg rat Fx1A in complete adjuvant. Onset of proteinuria was usually observed 60 to 75 days after immunization; it revealed the characteristic features found by immunofluorescence, histopathology, and electron microscopy. Apparently, such minute doses of a crude tissue fraction probably containing less than 5 percent of the specific nephritogenic antigen (*20*) do not quantitatively account for a significant part of the deposited antigen. We found that probably less than 0.025 mg of specific antigen is required for induction of the disease. It has been estimated that 0.2 to 0.5 mg of antigen is bound in the glomeruli of a rat in well-established experimental allergic glomerulonephritis (*10*). Thus, the immunizing antigen cannot quantitatively account for all of the tubular antigen found in the glomerular deposits, and the deposited antigen must be predominantly of autologous origin.

A nonglomerular antigen, normally occurring in the apex and brush border of proximal renal tubular epithelium, is clearly present in the disease in apparent association with γ-globulin of the host and complement in a granular fashion along the glomerular basement membrane in a pattern typical for immune-complex nephritis. When the disease is produced by immunization with large amounts of antigen, both autologous and exogenous antigens are deposited in the glomeruli; but when the typical disease is produced with a small immunogenic challenge, most of the antigen participating in complex formation and depositing in the glomeruli must be autologous. These observations establish the validity of the autologous immune-complex pathogenesis of allergic glomerulonephritis induced by TA. Three salient features are intrinsic to the autologous immune-complex concept. (i) The disease must be mediated by circulating antigen-antibody complexes; (ii) the antigenic constituent of the complexes is of autologous origin; and (iii) the host produces antibody capable of reacting with an autologous antigen unrelated anatomically or immunologically to the site of injury. Autologous immune-complex diseases are then distinguishable from those autoimmune diseases which are the result of direct interaction of antibody with a specific antigen constituent of the site of injury.

THOMAS S. EDGINGTON
RICHARD J. GLASSOCK*
FRANK J. DIXON
Department of Experimental Pathology, Scripps Clinic and Research Foundation, La Jolla, California

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* Present address: Kidney Laboratory, Peter Bent Brigham Hospital, Boston, Massachusetts.

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