capable of inducing both intestinal calcium transport and bone calcium mobilization. The 25-hydroxy derivative of 5, 6-*trans*-vitamin D_3 is unable to stimulate bone calcium mobilization while stimulating intestinal calcium transport (*7*).

It is also surprising that isovitamin D_3 is biologically active in normal rats but not in anephric rats. Although there are many possible explanations, it seems likely that a small amount of vitamin D_3 may have remained in the preparation. This small amount, which could go undetected by either thin-layer chromatography or gas-liquid chromatography, would be metabolized only in normal animals to 1,25-$(OH)_2D_3$ and elicit a response in both the bone and intestine. Another possible explanation is that in isovitamin D_3 the A ring may not be rotated 180°, and therefore the kidney is required for a hydroxylation on C-1 to provide an analog of 1,25-$(OH)_2D_3$ which is biologically active in the intestine and bone. This problem remains to be solved.

In any case, our results demonstrate that, in addition to the 5,6-*trans* isomers, another isomer of vitamin D_3—isotachysterol$_3$—can function in bone and intestine of anephric animals. The conversion of vitamin D_3 to the isotachysterol$_3$ is as simple to carry out as the 5,6-*trans* isomerization. However, it has the added advantage that the procedure results in virtually quantitative conversion of vitamin D_3 to the isotachysterol. Thus its preparation would be less expensive than that of 5,6-*trans*-vitamin D_3, and its purification before use would be considerably simpler. Its possible utility in cases of renal osteodystrophy, therefore, seems evident.

M. F. HOLICK, H. F. DELUCA
P. M. KASTEN, M. B. KORYCKA
Department of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin–Madison, Madison 53706

References and Notes

1. M. Horsting and H. F. DeLuca, *Biochem. Biophys. Res. Commun.* **36**, 251 (1969).
2. G. Ponchon, A. L. Kennan, H. F. DeLuca, *J. Clin. Invest.* **48**, 2032 (1969).
3. D. R. Fraser and E. Kodicek, *Nature* **228**, 764 (1970).
4. I. T. Boyle, L. Miravet, R. W. Gray, M. F. Holick, H. F. DeLuca, *Endocrinology* **90**, 605 (1972); M. F. Holick, M. Garabedian, H. F. DeLuca, *Science* **176**, 1146 (1972); R. G. Wong, A. W. Norman, C. R. Reddy, *J. Clin. Invest.* **51**, 1287 (1972).
5. A. S. Brickman, J. W. Coburn, A. W. Norman, *N. Engl. J. Med.* **287**, 891 (1972).
6. E. J. Semmler, M. F. Holick, H. K. Schnoes, H. F. DeLuca, *Tetrahedron Lett.* **40**, 4147 (1972).
7. M. F. Holick, M. Garabedian, H. F. DeLuca, *Biochemistry* **11**, 2715 (1972).
8. ———, *Science* **176**, 1247 (1972).
9. H. H. Inhoffen, K. Bruckner, R. Grundel, *Chem. Ber.* **87**, 1 (1954).
10. T. K. Murray, K. D. Day, E. Kodicek, *Biochem. J.* **98**, 293 (1966).
11. Supported by PHS grants AM-14881 and AM-15512.

26 January 1973; revised 9 March 1973

Transfer of Experimental Autoimmune Renal Cortical Tubular and Interstitial Disease in Guinea Pigs by Serum

Abstract. *Guinea pigs injected with rabbit tubular basement membranes and Freund's adjuvant develop progressive renal cortical tubulointerstitial disease and deposit autoantibodies in their cortical tubular basement membranes. The identical, even fatal, disease may be produced in normal guinea pigs by a single intraperitoneal injection of serums obtained from guinea pigs with this tubulointerstitial disease, provided such serums contain sufficient amounts of autoantibodies against tubular basement membranes.*

Guinea pigs injected with heterologous renal basement membranes and complete Freund's adjuvant develop renal cortical tubular disease and form antibodies, some of which react with their own cortical tubular basement membranes (TBM) (*1*). Severe cortical tubulointerstitial disease is correlated with extensive linear fixation of antibodies along the cortical TBM and high titers of serum autoantibodies to TBM (*1, 2*). We now report the induction of progressive and fatal renal tubulointerstitial disease in normal guinea pigs by intraperitoneal injection of serum containing large amounts of autoantibodies to TBM.

Preparations rich in rabbit TBM were emulsified in complete Freund's adjuvant (*1*). On days 1 and 14 donor guinea pigs (*3*) were injected with 0.1 ml of emulsion [15 mg of TBM (wet weight) per milliliter of emulsion] intradermally in six sites on the back. Control donors were either injected with an emulsion of complete Freund's adjuvant alone or were untreated. All donors were exsanguinated on day 21. The titers of antibodies against guinea pig TBM or glomerular basement membrane (GBM) in the serums of 125 donors with tubular disease were determined by indirect immunofluorescence (*4*). Three pools of serums were formed; in each pool the ratio of the titer of antibody to TBM to the titer of antibody to GBM was different (Table 1). The pooled serums were sterilized by passage through an autoclaved 0.45-μm membrane filter.

A total of nine recipients was injected intraperitoneally with donor serum. Prior to the injections of donor serums, one kidney was removed from each of two recipients to serve as a control for the remaining kidney in pathologic and immunologic studies. The progression of disease was studied in five recipients, after injection of donor serums, by removing one kidney from each of two recipients on day 2 and from each of the other recipients on days 3, 7, and 8 and comparing it with the contralateral kidney subsequently obtained at autopsy. Donor serum was injected into two recipients with both kidneys intact. Blood urea nitrogen (BUN) tests and urinalyses were performed daily (*5*). Four uremic recipients were killed when moribund on days 10 to 14 (Table 1). The remaining recipients were killed on day 14. Renal tissue from nephrectomy or autopsy was prepared by conventional methods for light and fluorescence microscopy. Cryostat sections (4 μm) of recipient and donor kidneys were studied by direct immunofluorescence for deposition of immunoglobulin G (IgG) (*6*) and by indirect immunofluorescence for detection of complement (C3) (*7*). Recipient kidneys were eluted with citric acid buffer at *p*H 3.2 (*1*). These eluates and daily serum samples were layered over cryostat sections of homologous kidney or of those kidneys that were removed before injection of the recipient and stained with fluorescein-conjugated rabbit antiserum to guinea pig IgG to identify donor antibodies which can react with TBM or GBM.

All nine recipients developed progressive cortical tubulointerstitial disease (Table 1). Fourteen kidneys were examined 2 to 14 days after injection of donor serum. The development of disease was correlated directly with extensive deposition of IgG along the TBM in vivo (*8*). The autoantibodies to TBM injected were in large excess, since they were still detected in the serums of all nine recipients for 10 to 14 days after transfer. Control recipients had no evidence of autoantibodies to

TBM in their kidneys or serums and had no evidence of cortical tubular disease (Table 1).

The immunologic specificity of the serum and specificity of tissue damage in the recipients was shown by absorption. After absorption of a potent serum (Table 1, pool B) with washed whole guinea pig kidney homogenate, the titer of autoantibodies to GBM fell from 1 : 80 to zero and that of autoantibodies to TBM fell from 1 : 640 to 1 : 5. Recipients of this absorbed serum did not develop disease, and IgG was not seen along the TBM (Table 1). However, when we absorbed this potent serum with guinea pig GBM, the titer against GBM fell to zero, but the titer against TBM remained at 1 : 640. Recipients of this absorbed serum had extensive deposition of IgG along the TBM, as shown by immunofluorescence staining, and developed tubulointerstitial disease (Table 1). Further, autoantibodies to TBM were found in acid eluates of these kidneys. These experiments confirm that the transfer of tubulointerstitial disease is dependent on the transfer of sufficient amounts of donor autoantibodies to TBM; moreover, serums with or without autoantibody to GBM produced on transfer the identical tubulointerstitial nephritis (*9*).

Table 1. Serum transfer of experimental autoimmune renal tubulointerstitial disease. Abbreviation: CFA, complete Freund's adjuvant.

Donor					Recipients (No. positive/No. injected)			
Immunization	Serum pool	Serum* (ml)	Autoantibody titer to:		Linear fixation of IgG along:		Renal tubulointerstitial disease	Mortality
			GBM	TBM	GBM	TBM		
Rabbit TBM and CFA	A	20–50	1 : 20	1 : 640	4/4	4/4	4/4	3/4†
	B	32	1 : 80	1 : 640	2/2	2/2	2/2	1/2‡
	C	65	1 : 160	1 : 160	1/1	1/1	1/1	0/1
	B§	25	0	1 : 640	±/2	2/2	2/2	0/2
Controls for serum transfer								
Rabbit TBM and CFA	B‖	45	0	1 : 5	±/2	0/2	0/2	0/10
Untreated	D	20–50	0	0	0/4	0/4	0/4	
CFA only	E	20–50	0	0	0/4	0/4	0/4	

* Animals received a total of 20 to 32 ml in one injection. Those animals receiving a larger volume were given two divided injections 24 hours apart. † Pool A: Of the three animals that died, two had one kidney removed on days 3 and 8 and died in uremia on days 13 and 12; the third had both kidneys until it died in uremia on day 13. The one that did not die had both kidneys. ‡ Pool B: The animal that died had its first kidney removed on day 7 and was moribund on day 10. § Pool B (25 ml) was absorbed with guinea pig GBM [350 mg (wet weight)] until the titer against GBM was 0. ‖ Pool B (45 ml) was absorbed with 4.1 g of repeatedly washed whole guinea pig kidney homogenate containing GBM and TBM.

The earliest kidney examination was 2 days after transfer. A few focal lesions consisted of several damaged proximal tubules, close to a glomerulus, in the outer third of the cortex. Degenerative, necrotic, regenerative, or proliferative changes were seen in the tubular epithelium. Nuclear debris or inflammatory cells were in tubular lumina or in interstitium, and some peritubular capillaries were congested. The affected tubules were surrounded by mononuclear cells, most of which appeared to be macrophages. These cells were in intimate contact with the TBM, and in some cases seemed to be actually destroying the TBM or invading the tubules (Fig. 1). Some macrophages seemed to fuse to form giant cells which surrounded all or part of a tubule. As new focal lesions appeared, the entire two-thirds of the outer cortex became severely involved, whereas the third of the cortex nearest the medulla was less affected. A few polymorphonuclear cells and some nuclear debris were in the interstitium at any stage. Mitotic divisions indicated active cellular proliferation of tubular epithelium and interstitial mononuclear cells. Once tubular damage started, the cortical area became progressively damaged each day.

By direct immunofluorescence, on day 2, autoantibodies to TBM were present in a smooth linear pattern along the entire circumference of the TBM of 80 to 90 percent of the cortical proximal tubules. The macula densa and distal tubules stained faintly, if at all. As the disease progressed, fluorescent staining for IgG along the TBM became disrupted or disappeared. Fluorescent staining for complement (C3) was similar to that for IgG. The remaining intact tubules were separated, presumably by interstitial cells, giant cells, edema, or damaged tubular remnants.

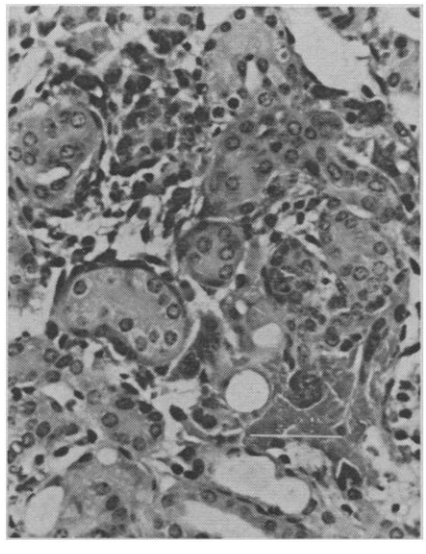

Fig. 1. Kidney section obtained by nephrectomy from a recipient guinea pig 8 days after injection of donor serum. The animal died in uremia on day 12. Degenerative, necrotic, and regenerative changes are in the tubular epithelium. Mononuclear cells (mostly macrophages and some lymphocytes), giant cells, hemorrhage, and remnants of damaged tubules are present. Macrophages and giant cells intimately surround the tubules and are phagocytosing them. These changes are identical to those in actively immunized animals (hematoxylin and eosin stain; the scale represents 50 μm).

The fluorescent staining for IgG along the TBM is present before there is any accumulation of peritubular mononuclear cells. Later on, the disruption of the fluorescent staining for IgG along the TBM begins and increases with time. This suggests a lag in time after deposition of antibodies in the TBM before peritubular macrophages begin to accumulate and significantly disrupt the TBM or destroy tubules. Hence, by all criteria, the clinical, pathologic, and immunopathologic disease in the recipients was remarkably identical to that of the donor (*10*).

Our results show that (i) serum free of cells and containing sufficient amounts of autoantibodies to TBM can transfer this autoimmune renal tubular disease; (ii) one injection of enough serum can produce the identical and even fatal disease; (iii) absorption of potent serum with whole guinea pig kidney homogenate, but not guinea pig GBM, removed almost all antibodies to TBM and made the serum incapable of transferring disease; (iv) macrophages and giant cells appear after the deposition of autoantibodies to TBM along the TBM and later invade and destroy the TBM and tubules.

Thus, autoantibodies to TBM are essential in the pathogenesis of this new autoimmune renal tubular and

interstitial disease. These antibodies appear to fix C3. Mononuclear cells (predominantly macrophages) surround the tubules almost simultaneously with signs of tubular cell damage. Activated macrophages are present (*11*). Antibodies may be necessary for macrophages to effectively phagocytose the TBM and tubules (*12*).

We thus have developed two contrasting models of autoimmune renal damage. In sheep, autoantibodies to GBM damage glomeruli (*13*). Mononuclear cells were not involved. In guinea pigs autoantibodies to TBM selectively damage tubules and interstitium but not glomeruli. Mononuclear cells participate in the progressive tissue injury initiated by autoantibodies to TBM. Thus, the pathogenesis of these two diseases is different. The specificity of these autoantibodies and tissue injury indicate that unique target antigens are in the GBM and TBM.

The serum transfer induces fatal disease in the recipients and reproduces in the recipients all of the cellular manifestations present in the disease of the donor. The transfer of cell-free serum seems to exclude delayed-type cellular hypersensitivity as a mechanism for mononuclear cell participation. Hence, the serum transfer should provide a useful, easy means for studying a new and fatal mechanism of tissue injury initiated by autoantibodies to TBM and involving mononuclear cells, and for studying humoral-cellular interactions in vivo.

This new model of renal tubulointerstitial disease may be applicable to the study in man and domestic animals of tubulointerstitial disease which may be superimposed on glomerular lesions (*14*), renal homograft reactions (*15*), and certain kinds of tubulointerstitial diseases whose pathogenesis is presently obscure.

RAYMOND W. STEBLAY
ULRICH RUDOFSKY
New York State Kidney Disease Institute and *Department of Pathology, Albany Medical College, Albany, New York 12208*

References and Notes

1. R. W. Steblay and U. Rudofsky, *J. Immunol.* **107**, 589 (1971); R. W. Steblay, *Fed. Proc.* **29**, 237 (1970). The cortical tubular disease is characterized by a linear continuous deposit of autoantibodies along the cortical TBM, by degeneration, necrosis, and regeneration of tubular epithelium, by destruction of tubules and TBM, and by peritubular mononuclear cells, giant cells, interstitial hemorrhage, occasional nuclear debris, and polymorphonuclear cells. Animals develop glucosuria, proteinuria, hemoglobinuria, and uremia and die in acute or chronic renal disease.
2. We have found (*1*) animals with severe tubulointerstitial disease and no evidence of antibodies to GBM. Therefore, the incidence of renal tubular disease was correlated only with the deposition of autoantibodies to TBM and was independent of whether or not antibodies to GBM were found.
3. All guinea pigs (Albany strain) weighed 350 to 450 g and were obtained from the New York State Department of Health.
4. A. H. Coons, *Gen. Cytochem. Methods* **1**, 399 (1958). The titers of serum antibodies were the greatest dilution of serum that still gave positive staining along the TBM or GBM by indirect immunofluorescence.
5. Glucose, protein, and hemoglobin were tested in urine with Labstix (Miles Laboratory, Elkhart, Indiana).
6. Fluorescein-conjugated rabbit antiserum to guinea pig IgG was prepared as in (*1*).
7. Rabbit antiserum to complement (C3) was donated by Dr. M. M. Mayer, Johns Hopkins University. Fluorescein-conjugated goat antiserum to rabbit IgG was purchased from Hyland Laboratories, Los Angeles, and absorbed with guinea pig IgG before use.
8. In preliminary experiments, if insufficient amounts of donor autoantibodies to TBM were injected, little or no deposition of autoantibodies to TBM occurred, and no significant tubular disease developed (see also controls in Table 1).
9. In these experiments, neither donors nor recipients had detectable glomerular damage. Moreover, the amount or kinds (or both) of autoantibodies to GBM present did not influence the course of the tubulointerstitial disease. Under different experimental conditions, we could produce a diffuse, progressive, and fatal glomerulonephritis in guinea pigs.
10. The disease in both donors and recipients was characterized by a linear deposit of autoantibodies along the cortical TBM, by tubular damage, and by interstitial changes [as described in the text and (*1*)]. By day 21, 25 percent of donors were moribund; approximately 50 to 60 percent had ecchymoses, glucosuria, proteinuria, and hemoglobinuria; and in 40 to 50 percent the BUN was greater than 75 mg per 100 ml. By the 14th day, 4 of 9 recipients were moribund, 6 of 9 had ecchymoses, 8 of 9 had glucosuria, 6 of 9 had proteinuria, and 4 of 9 had hemoglobinuria, with a BUN greater than 75 mg per 100 ml.
11. Cytochemical stains for β-glucuronidase and acid phosphatase were done by Dr. R. Wylie, Johns Hopkins University.
12. D. S. Nelson, *Macrophages and Immunity* (Elsevier, New York, 1969); in *Mononuclear Phagocytes*, R. Van Furth, Ed. (Davis, New York, 1969). Additional possibilities for humoral-cellular interaction are numerous; they include anti-TBM antibodies acting as opsonins or cytophylic antibodies for macrophages. Macrophages may have receptor sites for the immune complex consisting of TBM, antibody to TBM, and complement. Other humoral factors in donor serum or host may facilitate the emigration, proliferation, and phagocytosis of macrophages, and giant cell formation.
13. R. W. Steblay, *J. Exp. Med.* **116**, 253 (1962); R. A. Lerner and F. J. Dixon, *ibid.* **124**, 431 (1966); R. W. Steblay and U. Rudofsky, *Nature* **218**, 269 (1968).
14. We found linear staining for IgG and C3 along the cortical TBM in glomerulonephritis in sheep [R. W. Steblay and U. Rudofsky, *Science* **160**, 204 (1968)] and in rats, and identified autoantibodies to TBM in rats [R. W. Steblay and U. Rudofsky, *Fed. Proc.* **28**, 426 (1969)].
15. G. M. Williams, H. M. Lee, R. F. Weymouth, W. R. Harlan, K. R. Holden, C. M. Stanley, G. A. Millington, D. M. Hume, *Surgery* **62**, 204 (1967).
16. Supported by PHS grant HL-13389. We thank Drs. C. Pirani and W. Kaufman for assistance.

4 December 1972; revised 12 March 1973

Radioreceptor Assay for Prolactin and Other Lactogenic Hormones

Abstract. *A radioreceptor assay with a sensitivity of 5 nanograms per milliliter has been developed for mammalian and avian pituitary prolactin, placental lactogenic hormones, and human growth hormone, using a membrane receptor preparation isolated from rabbit mammary glands. Prolactin preparations inhibited the binding of [^{125}I]prolactin to receptors in direct proportion to the biological potency of these preparations. Thus, the radioreceptor assay provides a convenient and simple assay for hormones which have lactogenic activity.*

The currently available prolactin bioassays, namely, the pigeon crop sac assay (*1*) and the in vitro rabbit and mouse mammary gland assays (*2*), while adequate, are difficult to perform, time-consuming, and costly. With the demonstration that prolactin linked to Sepharose is biologially active in mouse mammary tissue in vitro (*3*) and that ovine [^{125}I]prolactin is taken up by rabbit mammary tissue in vitro and in vivo (*4*), it appeared feasible to develop a membrane receptor assay for prolactin, as has been done for other polypeptide hormones (*5*). Indeed, the successful development of such an assay by using membranes obtained from midpregnant mouse mammary tissue has been presented (*6*). The same group of investigators subsequently reported that their initial findings with regard to sensitivity of their assay were not reproducible (*7*). In view of the limited amount and the heterogeneity of mammary tissue which can be obtained from a pregnant mouse, we turned our attention to the midpregnant rabbit mammary gland as a source of membrane receptors. Previously we had demonstrated that midpregnant rabbits injected intramuscularly with 10 mg of human placental lactogen and 5 mg of hydrocortisone daily for 4 days exhibited a remarkable development of their mammary glands (*8*). An average of 100 g of fresh mammary tissue can readily be dissected from one rabbit treated in this manner. Moreover, histo-