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**Research Article**

Factor D (D) is an essential component of the alternative complement pathway. To determine whether D is catabolized by the kidney and, if so, at what site, we studied the renal handling of human D by in vivo nephron microperfusion and in vitro perfusion of rat kidneys. Human D was purified and labeled with 125I. Individual nephrons were perfused in vivo at varying rates with perfusate that contained 125I-D and [14C]inulin. When nephrons were perfused from proximal sites with perfusate 125I-D in a concentration of 3.0 micrograms/ml, urinary recovery of 125I-D increased (P less than 0.05) from 57.7 +/- 5.0 to 74.4 +/- 2.5% as tubule fluid flow rate was increased from 10 to 40 nl/min; recovery of 125I-D was less than (P less than 0.001) [14C]inulin recovery at all perfusion rates. At 20 nl/min, an increase in perfusate 125I-D concentration from 1.5 to 3.0 micrograms/ml was associated with an increase (P less than 0.001) in urinary 125I-D recovery (42.1 +/- 4.0 vs. 65.8 +/- 2.6%). Similarly, the addition of unlabeled D, 30 micrograms/ml, to 125I-D, 3.0 micrograms/ml, increased urinary 125I-D recovery (95.3 +/- 2.1%) at 20 nl/min. When nephrons were perfused from early distal segments at 10 nl/min, 125I-D recovery (91.2 +/- 4.3%) did not differ from [14C]inulin recovery (95.8 +/- 1.3%). In the isolated perfused filtering kidney, the […]



# Human Complement Protein D Catabolism by the Rat Kidney

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## Abstract

**Factor D (D) is an essential component of the alternative complement pathway. To determine whether D is catabolized by the kidney and, if so, at what site, we studied the renal handling of human D by in vivo nephron microperfusion and in vitro perfusion of rat kidneys. Human D was purified and labeled with $^{125}$I. Individual nephrons were perfused in vivo at varying rates with perfusate that contained $^{125}$I-D and [$^{14}$C]inulin. When nephrons were perfused from proximal sites with perfusate $^{125}$I-D in a concentration of 3.0 μg/ml, urinary recovery of $^{125}$I-D increased ($P < 0.05$) from 57.7±5.0 to 74.4±2.5% as tubule fluid flow rate was increased from 10 to 40 nl/min; recovery of $^{125}$I-D was less than ($P < 0.001$) [$^{14}$C]inulin recovery at all perfusion rates. At 20 nl/min, an increase in perfusate $^{125}$I-D concentration from 1.5 to 3.0 μg/ml was associated with an increase ($P < 0.001$) in urinary $^{125}$I-D recovery (42.1±4.0 vs. 65.8±2.6%). Similarly, the addition of unlabeled D, 30 μg/ml, to $^{125}$I-D, 3.0 μg/ml, increased urinary $^{125}$I-D recovery (95.3±2.1%) at 20 nl/min. When nephrons were perfused from early distal segments at 10 nl/min, $^{125}$I-D recovery (91.2±4.3%) did not differ from [$^{14}$C]inulin recovery (95.8±1.3%).**

**In the isolated perfused filtering kidney, the concentration of intact $^{125}$I-D in the perfusate declined 60.3±14.6% over 1 h. 83.4±6.3% of the decrement in $^{125}$I-D was catabolized by the kidney; the remainder was excreted in the urine as intact D. When glomerular filtration was prevented by increasing perfusate albumin concentration to 16 g/dl, perfusate intact ($^{125}$I-D) remained unchanged over 1 h.**

**These data show that human D is catabolized by the kidney via glomerular filtration and reabsorption by the proximal nephron. Reabsorption of D appears to be a saturable process.**

## Introduction

The complement system comprises a group of proteins that interact in a cascade to form biologically active fragments that are integrally involved in the inflammatory process (1). One of these proteins, factor D (D),[1] a serine protease with a molecular weight of 23,750 (2), participates in the formation of C3 convertase in the initiation sequence and amplification loop of the alternative pathway (3, 4). The sites of catabolism of this and other proteins of the complement system have been unknown. Recently, Sturfelt et al. (5), and Volanakis et al. (6) demonstrated that serum D concentration correlates positively with serum creatinine concentration. We also showed that, in one patient with the Fanconi syndrome, urinary D concentration was extremely high (1,300 μg/dl compared with undetectable concentrations in normal individuals) (6). These data suggest that the kidney plays a major role in the removal of D from the circulation. Because in vitro alternative complement pathway kinetics is directly correlated with D concentrations (3), acceleration of complement activation that may occur in patients with renal failure may be an important contribution not only to progression of renal dysfunction, but also to some of the complications of end-stage renal disease.

Many low molecular weight proteins are known to be filtered at the glomerulus and subsequently reabsorbed and catabolized by the proximal tubule (7–10). Using both in vivo microperfusion and isolated perfused kidney techniques in the rat, the present series of experiments was designed to: (*a*) demonstrate directly catabolism of human D by the kidney; (*b*) determine the locus of uptake of D in the nephron; and (*c*) evaluate luminal factors that might influence reabsorption of D.

## Methods

*Purification and radiolabeling of D.* D was purified from either normal human plasma or urine from a patient with the Fanconi syndrome (6) by a combination of ion-exchange chromatography on Bio-Rex 70 and gel filtration on Bio-Gel P-60 (Bio-Rad Laboratories, Richmond, CA) followed by hydroxylapatite and reverse-phase high pressure liquid chromatography as described previously (2). Purity was confirmed by electrophoresis on 5–20% polyacrylamide gradient slab gels containing 0.1% sodium dodecyl sulfate (SDS-PAGE), using the discontinuous buffer system described by Laemmli (11). Protein bands were stained with silver nitrate as described by Merril et al. (12). Purified D was radiolabeled with $^{125}$I using the chloramine T method (13). 20 μl of D (300 μg/ml) were mixed with 10 μl 0.5 M sodium phosphate buffer, pH 7.5, 500 μCi $^{125}$I-Na, and 10 μl chloramine T (5 mg/ml in 0.5 M phosphate buffer, pH 7.5) in that order. The reaction mixture was incubated for 3 min at room temperature and the reaction was stopped by adding 75 μl sodium metabisulfite (2 mg/ml) and 75 μl potassium iodide (2 mg/ml); this was followed by extensive dialysis against 0.15 M NaCl. The specific radioactivity of $^{125}$I-D varied between 2.0 and 8.0 μCi/μg. ~99% of the radioactivity was precipitated by 10% trichloroacetic acid (TCA) and 92% by rabbit anti-D serum in the presence of Cowan I strain of *Staphylococcus aureus* (Bethesda Research Laboratories, Gaithersburg, MD).

*Microperfusion preparation.* Male Sprague-Dawley rats (Charles River Breeding Laboratories, Inc., Wilmington, MA), which weighed 255–300 g (274±4) and were maintained on regular rat chow (Ralston Purina Co., St. Louis, MO) and tap water ad libitum, were anesthetized with thiobutabarbital (Inactin, Promonta, Hamburg, Federal Republic of Germany), 100 mg/kg body wt, intraperitoneally. The animals were

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1. *Abbreviations used in this paper:* C3, third component of complement; D, factor D; % $D_{cat}$, amount of intact $^{125}$I-D catabolized by the kidney; % $D_{exc}$, fraction of intact $^{125}$I-D lost in the urine; $D_{lost}$, amount of intact $^{125}$I-D lost from the perfusate with time; $FR_D$, fractional recovery of $^{125}$I-D; $FR_{In}$, fractional recovery of [$^{14}$C]inulin; GSC, glomerular sieving coefficient; SDS-PAGE, sodium dodecyl sulfate polyacrylamide slab gel electrophoresis; U/P, ratio of urine to perfusate.

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placed on a servo-controlled heated table. Rectal temperature, which was maintained at 37°C, was monitored with a telethermometer (Yellow Springs Instrument Co., Yellow Springs, OH). Tracheostomy was performed and a PE-50 polyethylene catheter was placed in the right external jugular vein for intravenous infusion of 0.15 M NaCl at 3.0 mg/100 g body wt/h. A PE-50 polyethylene catheter was inserted into the right femoral artery for continuous monitoring of arterial pressure, which was measured with a model P23ID pressure transducer (Gould-Statham Instruments, Hato Rey, PR) and was recorded on a model 7D polygraph (Grass Instrument Co., Quincy, MA). A small, suprapubic incision was then made and the bladder was cannulated with a PE-50 polyethylene catheter. Through a left subcostal incision, the left kidney was exposed, gently separated from the perirenal fat and adrenal gland, and placed in a Lucite cup. The ureter was cannulated at the hilum with PE-50 polyethylene tubing. A well was formed on the surface of the kidney with 2% agar and was filled with water-equilibrated mineral oil.

After a 1-h equilibration period, a timed urine collection from the left kidney was made, urine volume was determined gravimetrically, and micropuncture was begun. A pipette filled with artificial tubule fluid, which contained 130 mM NaCl, 5 mM KCl, 1 mM $MgSO_4$, 10 mM $NaHCO_3$, 1 mM $NaH_2PO_4$, 1 mM $CaCl_2$, 4.5 mM urea, and was tinted with Food, Drug, and Cosmetic (FDandC) Green 3 (Keystone Aniline and Chemical Co., Chicago, IL), was inserted at random into an early proximal tubule segment close to a star vessel; a small bolus of the fluid was injected to outline the nephron segments. A pipette filled with bone wax was then inserted into the earliest surface proximal convolution or earliest surface distal segment and a small cast of wax 4–5 tubule diameters in length was injected into the tubule with a microdrive unit (Trent Wells, South Gate, CA). A pipette that was attached to a microperfusion pump (World Precision Instruments, Inc., New Haven, CT), previously calibrated in vitro, was inserted into the tubule just distal to the wax block and the remaining nephron was perfused for 10 min. This pipette was filled with 0.15 M NaCl that was tinted with FDandC Green 3 and contained [$^{14}$C]inulin (inulin-carboxyl [$^{14}$C], 2.3 mCi/g, New England Nuclear, Boston, MA), 217 μg/ml, and human $^{125}$I-D, the concentration of which was varied as described below. The perfusate was allowed to equilibrate in the pipette for 1 h before microperfusion was begun. Urine from the micropunctured kidney was collected during the final 5 min of perfusion. These urine collections were analyzed for $^{125}$I activity with a gamma scintillation counter (Model 1185, Searle Analytic, Inc., Des Plaines, IL) and for $^{14}$C activity with a liquid scintillation counter (Packard Tri-Carb, model 3255, Packard Instrument Co., Inc., Downers Grove, IL). At the end of each experiment, using the same micropipette and microperfusion pump, the pre-equilibrated perfusate that contained the $^{125}$I-D and [$^{14}$C]inulin was pumped into scintillation vials at 10, 20, 30, and 40 nl/min for 5 min. This maneuver allowed direct comparison of $^{125}$I-D and [$^{14}$C]inulin activities of the in vivo proximal and distal perfusions with the in vitro perfusion obtained at the same flow rate and also circumvents the problem of possible adsorption of D to the glass micropipettes.

Fractional recovery of $^{125}$I-D ($FR_D$) was calculated as: $FR_D$ = $^{125}$I activity of urine sample/$^{125}$I activity of in vitro sample × 100%.

Fractional recovery of [$^{14}$C]inulin ($FR_{In}$) was calculated as: $FR_{In}$ = $^{14}$C activity of urine sample/$^{14}$C activity of in vitro sample × 100%.

Urine collections were discarded if $FR_{In}$ was <80%.

The microperfusion protocols were varied in the following fashions to examine the variables of D uptake. To determine the locus of D uptake, nephrons were perfused either from proximal or distal sites at 20 or 10 nl/min, respectively, with perfusate containing $^{125}$I-D in a concentration of 3 μg/ml, which is ~1.5 times the normal human serum D concentration of 1–2 μg/ml (14, 15). This concentration was chosen to ensure adequate $^{125}$I activity in the urine during perfusion at the lowest flow rate. To evaluate the role of tubule fluid flow rate in modulating D uptake, the nephron was perfused from proximal sites at 10, 20, 30, or 40 nl/min with perfusate containing $^{125}$I-D at 3 μg/ml. The influence of luminal $^{125}$I-D concentration on D uptake was shown by perfusing the nephron from a proximal location at 20 nl/min with perfusate containing D in a concentration of either 1.5 or 3.0 μg/ml. Finally, to demonstrate the competitive inhibition of uptake of $^{125}$I-D by unlabeled D, we added unlabeled D, 30 μg/ml, to perfusate containing $^{125}$I-D, 3.0 μg/ml, and the nephron was perfused from a surface proximal site at 20 nl/min with this solution or with perfusate containing only $^{125}$I-D at 3.0 μg/ml.

*Isolated perfused kidney preparation.* To investigate the roles of glomerular filtration and basolateral uptake in D catabolism further, we used the isolated perfused kidney technique. The preparation and perfusion of isolated kidneys used in these studies are similar to those reported by us previously (16, 17). Male Sprague-Dawley rats, which weighed between 210 and 320 g (259±17) and were maintained on regular rat chow and tap water ad libitum, were anesthetized with an intraperitoneal injection of pentobarbital sodium (Nembutal, Abbott Laboratories, North Chicago, IL), 6–7 mg/100 g body wt. The abdominal cavity was exposed and the right ureter was cannulated with PE-10 polyethylene tubing. To maintain continuous renal perfusion, the superior mesenteric artery was cannulated with a beveled, 18-gauge needle that was advanced into the right renal artery and secured with ligatures. The right kidney was then excised from its surrounding tissues and was suspended from the cannulation needle while being perfused by gravity at 130 cm $H_2O$ for 30–60 s, before pump perfusion at 100 mmHg was begun. The perfusion pressure takes into account the pressure decline across the cannula. The kidney was perfused at 37–38°C in a water-jacketed, recirculating perfusion apparatus. The perfusing solution was oxygenated continuously with a mixture of 95% oxygen/5% carbon dioxide. The perfusate consisted of a blood-free, Krebs-Ringer bicarbonate buffer that contained 10 mM sodium acetate, 5 mM D-glucose, 5 mM urea, and 7.5% albumin (Bovuminar; Armour Pharmaceutical Co., Tarrytown, NY) in the normal, filtering preparations. To prolong viability of the isolated kidney preparation, the perfusate also contained the following L-amino acids: methionine, alanine, glycine, serine, proline, isoleucine, glutamine, arginine, and aspartic acid (18). The solutions were adjusted to pH 7.4 and the final osmolality was ~300 mOsm/kg. To stop glomerular filtration, perfusate albumin concentration was increased to 16 g/dl (19). Unlike other techniques that produce a nonfiltering kidney preparation (20), this method stops glomerular filtration without decreasing renal perfusion pressure.

[$^{14}$C]inulin (inulin-carboxyl [$^{14}$C], 2.3 mCi/g, New England Nuclear, Boston, MA), 45 μg/ml, was added to the perfusate at the start of the experiment. The kidney was discarded if the inulin clearance fell to <100 μl/min. Cessation of glomerular filtration in the nonfiltering preparation was confirmed by the absence of significant urine flow and by the failure to observe a fall in the concentration of radioactive inulin in the perfusing medium. 15–20 min after isolated perfusion was begun, human $^{125}$I-D, 12 ng/ml, was added to the perfusate. 1.5-ml samples of perfusate were obtained 5, 15, 30, 45, and 60 min after $^{125}$I-D was added. Urine samples were collected in preweighed vials every 15 min; urine volume was determined gravimetrically. Perfusate flow rate was measured by an in-line predictability flow-meter (Manostat Corp., New York, NY) and was adjusted to maintain arterial pressure at 100 mmHg in both filtering and nonfiltering preparations. Sodium and potassium concentrations in perfusate and urine samples were measured using a flame photometer (Model 343, Instrumentation Laboratory, Inc., Lexington, MA). $^{14}$C activities in perfusate and urine samples were determined with a liquid scintillation counter (Packard Tri-Carb, model 3255; Packard Instrument Co., Inc.).

Because of the recirculation of $^{125}$I in this closed-circuit perfusion system, loss of intact $^{125}$I-D from perfusate was determined by immunoprecipitating $^{125}$I-D from perfusate and urine samples with a monospecific rabbit anti-D serum. In a typical experiment, 250 μl of perfusate or 50–100 μl of urine, containing ~$10^4$ cpm, was mixed with 5 μl of anti-D serum and 50 μl of 10% wt/vol suspension of formalin-fixed Cowan I, *Staphylococcus aureus* (Bethesda Research Laboratories). The tubes were rotated at 4°C for 48–60 h. The cells were pelleted by centrifugation at 6,500 *g* for 5 min, washed twice with 750 μl of borate-buffered saline, pH 8.3, containing 0.2% human albumin and 0.5% NP-40 (Calbiochem-Behring Corp., La Jolla, CA), and counted in a gamma scintillation counter (Beckman Instruments, Inc., Fullerton, CA). Production and specificity of this rabbit anti-D antibody has been described previously (21). To allow time for equilibration, the activity of intact $^{125}$I-D of the

5-min sample was set at 100% in each experiment; subsequent intact $^{125}$I-D activities in the perfusate were then compared with this activity.

The amount of intact $^{125}$I-D lost from the perfusate with time, $D_{lost}$, was calculated as: $D_{lost} = ([^{125}I\text{-}D]_i - [^{125}I\text{-}D]_f) \times V_p$, where $[^{125}I\text{-}D]_i$ = initial perfusate $^{125}$I activity of intact D; $[^{125}I\text{-}D]_f$ = final perfusate $^{125}$I activity of intact D; and $V_p$ = perfusate volume.

The fraction of intact $^{125}$I-D lost in the urine, % $D_{exc}$, was calculated as: % $D_{exc} = [^{125}I\text{-}D]_u \times V_u/D_{lost} \times 100\%$, where $[^{125}I\text{-}D]_u$ = the total urinary $^{125}$I activity of intact D; and $V_u$ = the total urine volume.

Finally, the amount of intact $^{125}$I-D catabolized by the kidney, % $D_{cat}$, was calculated as: % $D_{cat}$ = 100% − % $D_{exc}$.

To verify that the immunoprecipitates contained only intact $^{125}$I-D and not low molecular weight fragments of $^{125}$I-D, SDS-PAGE was performed using 10–20% gradient polyacrylamide slab gels, 0.75 mm thick, and the buffer system described by Laemmli (11). Samples were prepared by suspending the pellets in 50 μl 0.06 M Tris-HCl, pH 6.8, containing 2% SDS and 5% β-mercaptoethanol. The gels were stained with Coomassie Brilliant Blue, dried, and exposed to X-Omat AR film (Eastman Kodak Co., Rochester, NY) at −70°C.

*Statistical analysis.* Values are given as mean±SE. Results were analyzed for statistical significance by the unpaired *t* test and analysis of variance where appropriate, with significance set at the 5% level.

## Results

*Microperfusion experiments.* Urinary flow rate from the left kidney averaged 10.5±3.2 μl/min. Urinary recovery of $^{125}$I-D (65.8±2.6%) was less than ($P < 0.001$) that of [$^{14}$C]inulin recovery (91.2±1.9%) from nephrons perfused with human $^{125}$I-D, 3.0 μg/ml, at 20 nl/min from early proximal segments (Fig. 1). In contrast, urinary recoveries of $^{125}$I-D (91.2±4.3%) and [$^{14}$C]inulin (95.8±1.3%) did not differ when perfused from distal sites. In addition, urinary recovery of $^{125}$I-D from proximal perfusions was less than ($P < 0.001$) recovery of $^{125}$I-D from distal perfusions.

To evaluate the factors modulating D uptake, nephrons were perfused from proximal sites at 10, 20, 30, or 40 nl/min. Urinary $^{125}$I-D recoveries were less than ($P < 0.001$) that of [$^{14}$C]inulin recoveries at all four perfusion rates; [$^{14}$C]inulin recoveries (93.3±2.9%; 91.2±1.9%; 91.0±3.2%; and 90.6±2.0%, respectively) were not different. Urinary $^{125}$I-D recoveries (57.7±5.0%; 65.8±2.6%; 68.2±4.2%; and 74.4±2.5%, respectively) increased progressively as perfusion rate increased with recovery, at 40 nl/min, being different from ($P < 0.05$) recovery of $^{125}$I-D at both 10 and 20 nl/min (Fig. 2). When the nephron was perfused at 20 nl/min with perfusate containing 1.5 μg/ml of $^{125}$I-D, urinary recovery of $^{125}$I-D decreased to 42.1±4.0% compared with 65.8±2.6%, which was the recovery of $^{125}$I-D at 3.0 μg/ml ($P < 0.001$) (Fig. 3). In addition, when unlabeled D, 30 μg/ml, was added to perfusate containing $^{125}$I-D, 3.0 μg/ml, and the nephrons were perfused from proximal sites at 20 nl/min with this solution, urinary $^{125}$I-D recovery increased to 95.3±2.1% (Fig. 3). Thus, although the protein we used was radio-iodinated, there was competition by unlabeled D for uptake, suggesting that the iodination process did not alter $^{125}$I-D recognition by the luminal membrane of the proximal tubule.

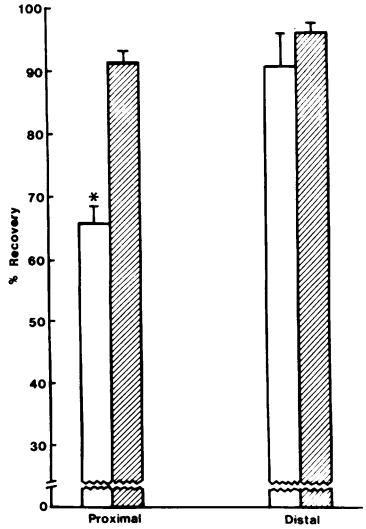


*Figure 1.* Comparison of urinary recoveries of $^{125}$I-D (□) and [$^{14}$C]inulin (■) from in vivo tubule perfusion from proximal or distal sites at 20 and 10 nl/min, respectively. Perfusate $^{125}$I-D concentration was 3.0 μg/ml. *$P < 0.001$ for $^{125}$I-D recovery from proximal vs. distal perfusion.

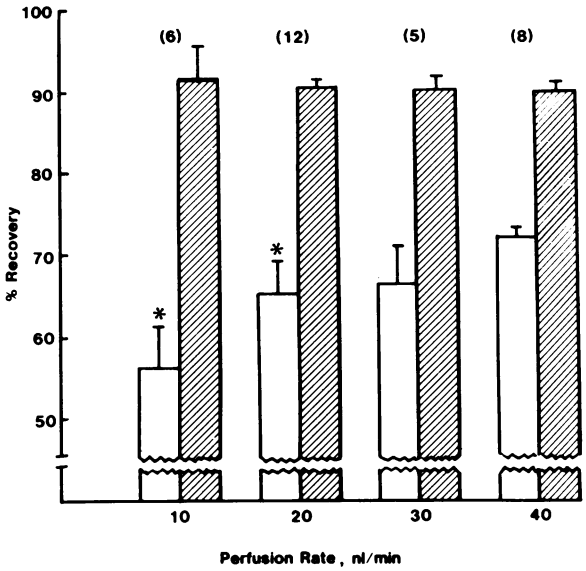


*Figure 2.* Urinary recoveries of $^{125}$I-D (□) and [$^{14}$C]inulin (■) during in vivo perfusion from a proximal location at varying perfusion rates. Perfusate $^{125}$I-D concentration was 3.0 μg/ml. *$P < 0.05$ compared with $^{125}$I-D recovery at 40 nl/min. Using a double-reciprocal plot of the Michaelis-Menten equation, the calculated maximum rate of D absorption, $J_D^{max}$, was 64.1 pg/min per nephron and the apparent Michaelis constant, $K_m$, was 123.4 pg/min per nephron. The number of observations made at each flow rate are shown in parentheses.

*Isolated perfused kidney experiments.* Urinary flow rate and inulin clearance were nil in the nonfiltering preparations, but renal perfusate flow was not different from flow in the filtering kidneys (Table I). In the filtering kidney experiments, the fractional reabsorption of sodium was 98.7±0.4% and the fractional excretion of potassium was 13.5±2.9%.

Because the perfusion system is a closed circuit, we examined $^{125}$I activity of intact $^{125}$I-D by immunoprecipitation. To demonstrate that the immunoprecipitate contained only intact D, we performed SDS-PAGE on every perfusate and urine sample from one nonfiltering and one filtering experiment. Fig. 4 shows the immunoprecipitates to be pure intact D. In the three nonfiltering kidneys, intact $^{125}$I-D concentration in the perfusate did not decline, whereas it did decline to 39.7±14.6% of baseline over 1 h in the six filtering preparations (Fig. 5). In the filtering kidney experiments, the ratio of urine to perfusate (U/P) intact $^{125}$I-D was 6.4±0.6, compared with a U/P inulin of 24.4±2.2; the fractional excretion of intact $^{125}$I-D ([U/P]D/[U/P]inulin) was 0.29±0.04. During perfusion of the nonfiltering kidneys, total $^{125}$I activity and the fraction of the total $^{125}$I activity that was immunoprecipitable (intact $^{125}$I-D) did not change, while

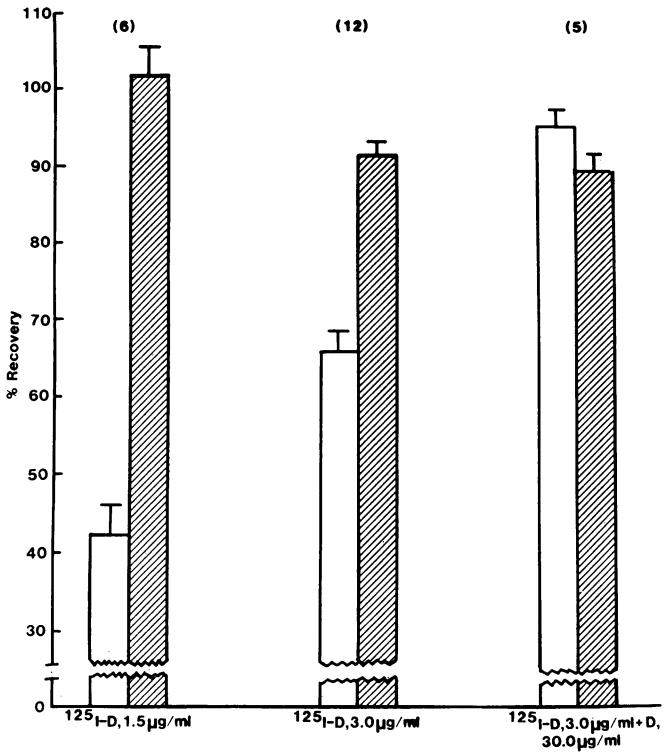


*Figure 3.* Effect of changing luminal D concentration on urinary recovery of $^{125}$I-D during in vivo nephron perfusion from proximal sites at 20 nl/min. As luminal D concentration was increased, D recovery increased, suggesting saturation kinetics. Number of observations is indicated in parentheses over each pair of bars. □, $^{125}$I-D; ■, [$^{14}$C]inulin.

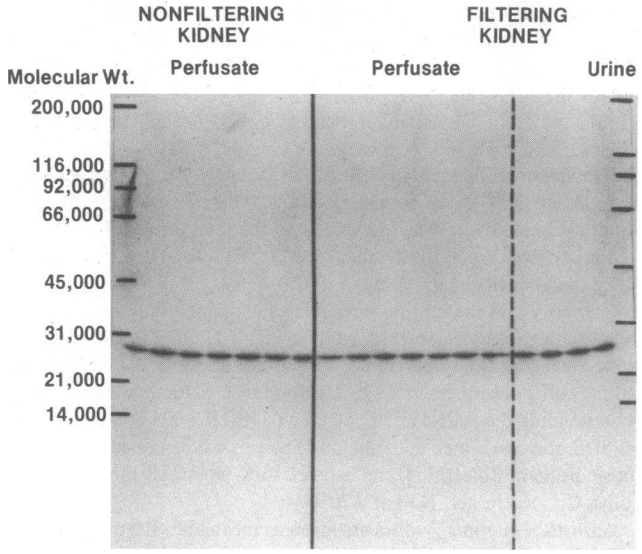


*Figure 4.* Representative SDS-PAGE patterns of immunoprecipitated intact $^{125}$I-D from perfusate and urine samples of nonfiltering and filtering isolated kidney preparations. The immunoprecipitated specimens are pure labeled intact D.

both total $^{125}$I activity and the fraction of this activity attributed to intact $^{125}$I-D in the perfusate declined over the course of every experiment that utilized a filtering kidney (Table II), indicating continued catabolism of intact $^{125}$I-D and removal of the catabolic products from the perfusate. Intact $^{125}$I-D in the urine of the filtering preparations was 72.2±7.1% of the total urinary $^{125}$I activity. Of the calculated loss of intact $^{125}$I-D from the perfusate over 1 h, 16.6±6.3% was excreted in the urine as intact $^{125}$I-D and 83.4±6.3% was catabolized.

## Discussion

The present study was undertaken to characterize further the role of the kidney in the metabolism of human complement factor D. The data show that: (*a*) D is filtered by the glomerulus and reabsorbed by the proximal tubule, (*b*) basolateral (peritubular) uptake of D does not occur, (*c*) D uptake is a saturable process, and (*d*) D is catabolized by the rat kidney.

Many low molecular weight proteins are known to be catabolized by the kidney. Thus, enzymes such as lysozyme and horseradish peroxidase (7); albumin (9, 10); immunoproteins such as light chains of immunoglobulins; and peptide hormones including insulin, growth hormone, parathyroid hormone, glucagon, and prolactin (7, 8, 20) are catabolized at least in part by the kidney. Uptake by renal epithelial cells can occur via two possible routes: glomerular filtration with subsequent reabsorption and basolateral uptake from the peritubular circulation.

Most peptides require glomerular filtration for their catabolism (7, 10). Molecular size, tertiary structure, and electrical charge are the major determinants of filtration of proteins (22), although proteins smaller than 25,000 mol wt usually have glomerular sieving coefficients (GSC) greater than 0.50 (7). Therefore, D, a globular protein that has a molecular weight of ~24,000 and isoelectric points of 6.6 and 7.0 (23), probably has a GSC of at least 0.50, making extraction of this protein from the circulation significant when the glomerular filtration rate is normal. We have previously shown a positive correlation between serum D and creatinine concentrations. In addition, one patient who had a Fanconi syndrome and a normal glomerular filtration rate had a urinary D concentration about 13 times the normal plasma concentration (6). Finally, in our present study, D concentration did not decline in the nonfiltering isolated perfused kidneys, but did decrease and was degraded in the filtering preparations. Taken together, we conclude that glomerular fil-

*Table I. Comparison of Initial Variables of Isolated Perfused Nonfiltering and Filtering Rat Kidneys*

| Preparation | *n* | Perfusate flow | Perfusion pressure | Inulin clearance | Urine flow |
|---|---|---|---|---|---|
| | | *ml/min per g kidney wt* | *mmHg* | *ml/min per g kidney wt* | *ml/min per g kidney wt* |
| Nonfiltering | 3 | 35±3.4 | 100 | 0.002±0.001* | 0.002±0.001* |
| Filtering | 6 | 36±2.7 | 100 | 0.407±0.067 | 0.011±0.002 |

* $P < 0.005$.

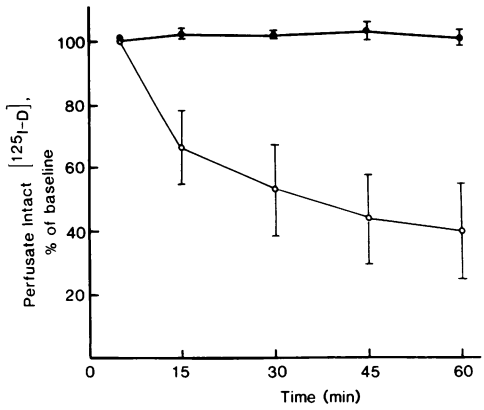


*Figure 5.* Loss of intact D over 1 h from perfusate of three nonfiltering and six filtering isolated kidney preparations. To allow time for equilibration, the $^{125}$I activity of the intact $^{125}$I-D concentration from the 5-min sample of each experiment was set at 100%. There was no demonstrable decline in $^{125}$I-D concentration when glomerular filtration was negligible (solid circles). In the normal, filtering preparations (open circles), $^{125}$I-D concentration fell 60.3±14.6% over 1 h.

tration is required for D catabolism and is a major regulator of the serum D concentration.

Basolateral (peritubular) absorption is the other route of protein uptake by the renal epithelium. Peritubular uptake of ferritin and horseradish peroxidase has been demonstrated with electron microscopic examination of isolated perfused proximal tubules (24) and with some hormones, including parathyroid hormone, glucagon, and insulin (8). However, despite renal perfusate flow and pressure not different from the isolated filtering kidneys, the nonfiltering kidneys did not catabolize D over 1 h. These observations suggest that peritubular uptake of D does not occur.

Filtered proteins are generally reabsorbed by the proximal tubule, although Madsen et al. (25) have shown uptake of ferritin by the rat distal convoluted tubule and Straus (26) demonstrated uptake of horseradish peroxidase into phagosomes in the distal tubule and collecting tubule. However, reabsorption of D by the distal nephron was not shown in our study. Except for small peptide hormones, such as angiotensin II and bradykinin, that may undergo hydrolysis along the luminal surface of the proximal tubule cells, low molecular weight proteins require endocytosis into the cell for catabolism (7, 9, 10). The initial adsorption of a protein to the luminal membrane is somewhat selective and related to the net charge and other structural features of the protein; reabsorption, at least for some proteins, is saturable (27, 28). In the present microperfusion study, we have shown that an increase in the load of D, either by increasing D concentration or tubule fluid flow rate, results in a decrease in D reabsorption by the proximal tubule. The quantity of labeled D that was used during these microperfusion experiments was insufficient to allow immunoprecipitation and subsequent analysis of the percentage of the total $^{125}$I activity in the urine that was due to intact $^{125}$I-D. Thus, we cannot state unequivocally that all of the $^{125}$I activity in the urine in the microperfusion experiments was intact D. However, luminal hydrolysis of proteins the size of D does not occur to a significant degree (7, 9, 10); our results neither confirm nor deny that endocytosis is the sole process of D catabolism by the proximal tubule. In addition, in the isolated perfused filtering kidney experiments, in which the concentration of D that was used was at least 100-fold greater than that used in the microperfusion experiments and the products of D catabolism were recirculated in the perfusate over an hour, 72% of the $^{125}$I activity in the urine was intact $^{125}$I-D. These data suggest that most of the urinary $^{125}$I activity in the microperfusion experiments was intact $^{125}$I-D. Assuming a normal human plasma D concentration between 1–2 μg/ml (14, 15) and GSC of ~0.50, the lowest luminal D concentration used in this study, 1.5 μg/ml, is only slightly in excess of the estimated luminal D concentration, 0.5–1.0 μg/ml. However, even at this concentration, $^{125}$I activity appeared in the urine. Taken together, our data suggest that the site of uptake in the rat proximal tubule has a relatively low affinity for human D and is readily saturated. Using the Michaelis-Menten equation to analyze the data obtained from the perfusions at 3.0 μg/ml (Fig. 2), we calculate a maximum rate of D absorption, $J_D^{max}$, of 64.1 pg/min per nephron and an apparent Michaelis constant, $K_m$, of 123.4 pg/min per nephron.

Although several studies have described the metabolism of complement proteins in health and in some disease states (29–31), the organs involved in any complement protein catabolism have not been identified. The present findings, together with our previous data (6), show that the kidney, or, in particular, glomerular filtration, is integral in maintaining serum D concentration in humans. Factor D circulates in human sera only as an active enzyme and can be recycled since it is not inhibited by known plasma protease inhibitors and it is not incorporated into the complexes formed by activation of the alternative complement pathway (3). In addition, selective removal of D activity totally impairs alternative pathway function; pathway activity is restored in a dose-dependent fashion by supplementation of the depleted sera with D (3). In the usual physiologic concentration of 1–2 μg/ml (14, 15), D is the rate-limiting enzyme in the alternative pathway; it becomes nonlimiting at nine times its normal level (3). Thus, glomerular filtration, an essential regulator of D concentration, may importantly modulate alternative pathway kinetics.

Many patients with end-stage renal disease have 10-fold or

*Table II. Comparison of Perfusate $^{125}$I Activity ([$^{125}$I]p) of In Vitro Perfused Filtering and Nonfiltering Rat Kidneys*

| Preparation | Final total [$^{125}$I]p* | Final intact [$^{125}$I]p* | Initial immunoprecipitable [$^{125}$I]p‡ | Final immunoprecipitable [$^{125}$I]p‡ |
|---|---|---|---|---|
| | *percent* | *percent* | *percent* | *percent* |
| Nonfiltering | 102.9±3.0 | 100.9±2.7 | 75.2±4.5 | 73.8±5.0 |
| Filtering | 59.7±10.4 | 39.7±14.6 | 74.4±3.9 | 43.2±10.6 |
| *P* | <0.025 | <0.025 | >0.4 | <0.05 |

* These percentages were derived from comparison of the activities of the final (60-min) perfusate samples with the initial (5-min) perfusate samples. ‡ These values represent the percentage of the total $^{125}$I activity in the 5-min and 60-min perfusate samples that is intact $^{125}$I-D.

greater elevations in D concentration (6) and theoretically would therefore have greatly accelerated kinetics of activation of the alternative pathway. The clinical implications of these findings are uncertain at present, but augmented complement activation may be involved in some hemodialysis-related syndromes. For example, activation of the alternative complement pathway by dialyzers composed of cuprophan membranes (32, 33) has been shown to cause leukopenia from pulmonary sequestration (34) and severe allergic reactions that include anaphylaxis with cardiopulmonary collapse (35). Determining the role of elevated D concentration in the pathogenesis of these syndromes may prove fruitful.

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