Nonfluorescent sperm from estrous uteri fertilized ova (that is, were capacitated), whereas fluorescent sperm from pseudopregnant uteri did not (noncapacitated). T-HCl-labeled sperm placed in the eye chamber, appendix, ileum or peritoneal cavity did not lose fluorescence. Presence or absence of fluorescence of labeled sperm placed in various regions and under different hormonal conditions correlates with what is known about where sperm will capacitate. Limited evidence with T-HCl-labeled human sperm compares with rabbit.

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Effect of Renin and Plasma Protein Loading on Albumin Catabolism in the Isolated Perfused Kidney.* (32291)

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It is generally accepted that in the normal kidney, plasma proteins are filtered at the glomerulus and are reabsorbed by the cells of the proximal tubules. However, little is known about how the kidney handles the reabsorbed proteins. We have shown that bilateral nephrectomy of the otherwise normal dog(1) or rat(2) does not significantly affect the rate of plasma albumin breakdown, leading to the conclusion that the normal kidney does not break down an appreciable amount of this plasma protein. Katz, Sellers and Bonorris(3) have shown that removal of the kidneys of rats made nephrotic and proteinuric by injection of aminonucleoside of puromycin or by the injection of nephrotoxic serum, results in at least a 50% reduction in the rate of plasma albumin catabolism. They have concluded that there was a correlation between the amount of proteinuria and the extent of albumin breakdown by the kidney. Finally, they used renin to produce a massive transient proteinuria in the rat, and noted a marked increase in albumin catabolism(4).

The experiments cited suggest a close relationship between proteinuria and albumin catabolism. By using the isolated perfused kidney preparation this relationship between proteinuria and albumin catabolism could be studied directly in the absence of all tissues. The rate of breakdown of I^{131} screened rabbit albumin has been studied in both the normal and the proteinuric perfused kidney. Proteinuria has been produced in the normal perfused kidney by administration of renin or by the elevation of the plasma protein concentration of the perfusion media. It was found that albumin catabolism was markedly increased in the perfused kidney during renin and protein loading proteinuria.

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Methods. The isolated rabbit kidney was perfused with whole heparinized rabbit blood as described in detail elsewhere(5). The kidney weighed 5-8 g, and the initial volume of circulating blood was about 200 ml. Two to seven microcuries of iodoalbumin were added 1/2 to 1 hour after the perfusion was started, and the experiments usually terminated 3 hours later. Blood and urine samples were taken at 20 to 30 minute intervals and deproteinized with 10% trichloroacetic acid (TCA). Creatinine, p-aminohippurate, protein and non-protein I^{131} activity determinations were made on all samples. The kidney was weighed at the end of the experiment, then homogenized in a known volume of 10% TCA, and the whole homogenate and the protein-free filtrate was assayed for I^{131} activity.

Preparaton of iodoalbumin and radioactive assay. Rabbit albumin was isolated from plasma following precipitation of the globulins by ammonium sulfate at a concentration of 28%. The supernatant was freed of salt by dialysis and the solution lyophilized. The albumin was further purified by the precipitation with zinc(6), the precipitate dissolved in excess Versene, and the solution lyophilized after thorough dialysis. Rabbit albumin, prepared by repeated alcohol fractionation, was also purchased from the Pentex Corp.

Albumin was first iodinated with cold iodine and then iodinated by the procedure of McFarlane(7). The yield as protein-bound iodine was 50-60% of the added activity. The non-protein activity was removed by deionization with Dowex-1 ion exchange resin. The albumin lots contained from 0.3 to 0.8 atoms of iodine per molecule of albumin. Albumins were assayed by disk electrophoresis and radioautography. To reduce the content of I^{131} activity not precipitable by TCA, the iodoalbumin preparations were dialyzed for 2-3 days against physiological saline. Such preparations had 0.1-0.3% of their activity soluble in TCA.

The I^{131} activity was assayed in a scintillation well counter in conjunction with a single-channel analyzer and scaler, as described previously(2).

Methods. Screening of iodoalbumin. This procedure which has been described previously (2), was first employed by MaFarlane(7) to remove any components that might have a half-life shorter than the bulk of the iodoprotein preparation. Rabbits injected with their homologous iodoalbumin were bled after 3-4 days and their plasma which contains the screened iodoalbumin was dialyzed for several days against physiological saline to remove the non-protein I^{131}.

Calculation of results. Albumin breakdown has been determined by using the methods previously described(2). The amount of albumin breakdown was computed from the initial total activity, the albumin concentration in the plasma and the non-protein I^{131} liberated during each period of the perfusion and recovered in the plasma and urine. The non-protein I^{131} activity recovered from the kidney at termination of the perfusion was divided by the number of periods and added equally to the plasma and urine I^{131} non-protein activity for each period. From the total amount of plasma albumin in the system, and the fraction of the iodoalbumin broken down during the periods, the rate of plasma protein breakdown was calculated and expressed as milligrams of albumin per gram of kidney per hour. For this calculation the initial and final plasma volume and albumin concentration for each period must be known. A protocol of a typical experiment is presented in Table I.

Results. The breakdown of iodoalbumin has been studied in the normal isolated perfused rabbit kidney and the proteinuric perfused kidney, where proteinuria has been produced by administration of 3.0 units of hog renin or by an increase in the plasma protein concentration of about 25% (12-44%), by addition of concentrated plasma protein(5). The results are summarized in Table II. Albumin breakdown is increased when proteinuria is produced by renin or plasma protein loading. A comparison of hourly breakdown rates in each group reveals that albumin breakdown is increased in the second hour, as compared to the first hour (control period), in all groups, but as can be seen in Table II the breakdown rates during the second hour of the renin and plasma protein loaded experiments are sig-

TABLE I. Calculation of Results.

Fraction	Vol, ml	I^{131} activity CPM/ml	I^{131} activity Total CPM	N.P. I^{131} CPM/ml	N.P. I^{131} Total CPM	N.P. I^{131} %	N.P. I^{131} % Net breakdown
Initial plasma:	124.1	18,244	2.264×10^6	13	1,613	.070	0
1st hr	120.2	17,380	2.089×10^6	19	2,281	.110	.04
2nd "	116.0	16,580	1.923×10^6	29	3,364	.175	.065
3rd or final hr	113.2	16,044	1.816×10^6	44	4,981	.274	.10
Urine:							
1st hr	2.40				1,853		
2nd "	1.70				1,473		
3rd "	1.55				1,677		
Kidney:			36,350		1,010		

Calculations:

Plasma N.P. I^{131}

1st hr $$\frac{(2.264 + 2.089) \times 10^6 \times .04}{2 \times 100} = 870$$

2nd hr $$\frac{(2.089 + 1.923) \times 10^6 \times .065}{2 \times 100} = 1{,}304$$

3rd hr $$\frac{(1.923 + 1.816) \times 10^6 \times .10}{2 \times 100} = 1{,}870$$

Non-protein activity/hr:

1st hr	Plasma —	870
	Urine —	1,853
	Kidney —	337
	Total	3,060
2nd hr	Plasma —	1,304
	Urine —	1,473
	Kidney —	337
	Total	3,114
3rd hr	Plasma —	1,870
	Urine —	1,677
	Kidney —	337
	Total	3,884

1st hr $$\frac{\text{Total N.P. } I^{131}}{\text{Albumin sp. act.} \times \text{wt kidney} \times \text{hr}} = \frac{3060}{550 \times 6.2 \times 1.0} = 0.90 \text{ mg/g/hr.}$$

nificantly higher than the first hour values for all groups, and are also significantly higher than the second hour values for the control group.

In 2 experiments, after the first control hour the perfusion pressure was reduced to 10 mm Hg for 10 minutes and then returned to the pre-ischemic perfusion pressure for studies to be carried out during the second (post-ischemic) hour. This was done in order to study the effect of ischemia on renal function, and particularly the effect of ischemia on urinary excretion of protein and the rate of iodoalbumin catabolism. Under these conditions increased proteinuria and albumin catabolism also develop (Table II).

Table II also lists 2 experiments in which the perfusion pressure was increased by 10-20 mm Hg, after the first control hour to study the effect in the subsequent hour of a non-specific diuresis and perfusion pressure elevation on N.P. I^{131} excretion and the calculation of albumin catabolism. While there is some increase in protein excretion, albumin catabolism is not increased.

Discussion. In earlier studies we have shown that the normal kidney does not play an important role in the catabolism of plasma albumin(2). Since that time it has been demonstrated that whereas the normal kidney does not catabolize an appreciable amount of plasma albumin, the kidney of the nephrotic and proteinuric rat does break down an appreciable amount of plasma albumin(3).

Further studies by this group showed that when a transient massive proteinuria was produced in the rat by administration of renin (4) there was a marked increase in albumin catabolism, leading to the conclusion that there was a correlation between amount of proteinuria and degree of albumin breakdown.

Our data support the findings of these investigators (3,4) and further demonstrate that when proteinuria is produced by the administration of renin or by plasma protein loading, a notable increase in plasma albumin catabolism occurs in an isolated perfused kidney in the absence of all tissues and substances of the body.

It is known that renin will cause a diuresis and an increase in pressure *in vivo* and indeed one finds the same response in the isolated perfused kidney preparation. To determine whether the increase in perfusion pressure was reponsible for the diuresis and the increase in urinary excretion of N.P. I^{131}, and therefore an increase in the calculated I^{131} albumin catabolism rate, the effect of raising the per-

TABLE II

	Albumin breakdown, mg/g/hr			Proteinuria, mg/hr		
	1st	2nd	3rd	1st	2nd	3rd
Controls	1.0	2.2		.3	.4	
	1.0	.8		1.6	.6	
	.5	.9	1.1	.6	.1	.04
	.9	1.0	1.2	4.5	2.4	2.7
	1.0	1.5	1.5	1.5	3.5	3.8
	1.1	1.2	1.4	4.5	3.9	4.2
Avg	.92	1.27		2.17	1.82	
SD ±	.22	.52		1.88	1.67	
SEM ±	.09	.21		.77	.68	
	P* = .2			P* = .8		
Conc. plasma protein	.6	2.6	1.7	4.2	12.5	8.8
	1.2	3.4	3.3	2.2	11.0	9.7
	1.2	1.8		2.0	10.9	
	1.2	1.8		2.0	6.3	
	1.0	2.9		2.0	5.5	8.0
	1.3	2.2				
	1.5	2.9		4.0	8.0	7.7
Avg	1.14	2.51		2.73	9.03	
SD ±	.29	.61		1.06	2.84	
SEM ±	.11	.23		.43	1.16	
	P* = <.001 P** = <.01			P* = <.001		
Renin	.8	2.7	1.8	1.3	11.5	2.1
	1.8	3.9	3.1	3.8	10.2	.5
	1.8	1.7		1.6	18.5	15.0
	1.0	2.8	2.2	1.4	37.9	19.2
	1.6	6.3		2.0	50.3	28.0
	.4	1.4		.6	15.8	14.8
	.7	1.3		1.9	50.2	
Avg	1.16	2.87		1.80	27.77	
SD ±	.57	1.77		.99	17.87	
SEM ±	.21	.67		.37	6.76	
	P* = <.05 P** = <.05			P* = <.01		
10′ ischemia	.7	2.5		1.3	3.2	
	1.2	3.8		4.1	16.8	
Increased perfusion pressure	2.1	1.7		.6	2.3	
	2.0	2.2		1.0	2.4	

P* A comparison of 2nd hr breakdown rates and proteinuria values to 1st hr values.
P** A comparison of 2nd hr breakdown rates of renin and plasma protein loading groups with 2nd hr breakdown rates of control group.

fusion pressure in the isolated kidney was also studied. It was found that an elevation of the perfusion pressure did produce a diuresis and an increase in excretion of N.P. I^{131}, as was observed after renin; but the plasma N.P. I^{131} level fell as compared to a marked rise in plasma N.P. I^{131} levels after renin, indicating that renin did increase plasma I^{131} albumin catabolism. Proteinuria was produced by raising the plasma protein concentration. This form of proteinuria was studied in order to rule out the enzyme effects of renin on some renal mechanism which might account for an increase in albumin breakdown unrelated to proteinuria *per se*. Protein loading experiments are not without serious disturbances to a steady state, particularly regarding the specific activity of the I^{131} albumin. However, it should be noted that protein loading produces a proteinuria which is predominantly an albuminuria, and an increase in the urinary excretion of N.P. I^{131} in spite of a decrease in the rate of urine flow. In addition, protein loading also causes an increase in the plasma N.P. I^{131} which is considerably higher than that observed in control studies.

Finally, when one compares the I^{131} albumin breakdown rates found in this study with the results we reported earlier(2), it is evident that we now find albumin breakdown values which are much higher. It should be noted that the function of the isolated perfused kidney during this earlier period was considerably inferior to the function of the perfused kidney used at this time. This comparison has been reported(5).

It may be that the higher rates of albumin catabolism observed in these perfusion studies (1.0 mg/g kid/hr) reflect both an increase in kidney function and an effect of the anoxia and trauma imposed upon the kidney in preparing it for perfusion. It has been suggested that the perfused kidney may even be elaborating a higher than normal amount of renin(8). When one compares the level of I^{131} albumin breakdown observed in our perfused kidney (1.0 mg/g kid/hr) with that reported by Reeve and Roberts(9) for the intact rabbit 230 to 318 mg/kg/24 hr, it is found by calculation that the two kidneys of our rabbit would breakdown approximately 14 mg/hr or 336 mg/24 hr. Further calculations would show that these kidneys come from 2-3 kg rabbits or that the kidneys are responsible for catabolizing 110 to 170 mg/kg b.w./24 hr. When this is compared to the data of Reeve and Roberts of 230 to 318 mg/kg b.w./24 hr, it can be seen that the kidney may be responsible for catabolizing a considerable portion of the total albumin catabolized by the rabbit. The breakdown rats observed in the perfused kidney fall within the rates observed in the intact rabbit and may very well reflect the capacity of kidney tissue to catabolize considerably higher amounts of albumin under conditions of trauma and/or proteinuria, which is also higher in the perfused kidney than in the normal rabbit(5).

Summary. Using the isolated perfused rabbit kidney preparation, the relationship between proteinuria and albumin catabolism was studied in the absence of all other tissues and humoral substances of the body. The rate of breakdown of I^{131} screened rabbit albumin was studied in both the normal and the proteinuric perfused kidney. Proteinuria was produced in the normal perfused kidney by administration of renin or by elevation of the plasma protein concentration of the perfusion media. It was found that albumin catabolism was markedly increased in the perfused kidney during renin and protein loading proteinuria.

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