# Factors Influencing the Handling of Insulin by the Isolated Rat Kidney

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ABSTRACT The renal handling of immunoreactive insulin was studied in the isolated perfused normothermic rat kidney to determine (*a*) the relative contributions of glomerular clearance and peritubular clearance to the renal clearance of insulin under different conditions, (*b*) what metabolic factors influence the ability of tubular cells to remove insulin from the glomerular filtrate and the peritubular circulation, and (*c*) whether the same factors influence the luminal and contraluminal uptake of insulin.

In control kidneys the organ clearance of insulin (OCi) was 974±63 μl/min (SEM), of which a maximum of 46% could theoretically be accounted for by filtration. OCi was not altered by fasting, lack of exogenous fuel (glucose), or the addition of cyanide. The glomerular filtration rate did not correlate with the OCi, but there was a significant ($P < 0.001$) negative correlation ($r = -0.828$) between the peritubular clearance and glomerular filtration rate. Both *N*-ethylmaleimide and cold (10°C) reduced the rate of insulin removal. Fractional excretion of filtered insulin (9.7±1.7% in controls) was not significantly altered by fasting or perfusing without glucose. In contrast, KCN increased fractional excretion of insulin to 41.9±3.7% whereas cold increased fractional excretion to 69.0±3.3%.

This study indicates that renal tubular cells remove insulin from the tubular lumen and the peritubular compartment. Furthermore, the data suggest that insulin removal by tubular cells is a temperature-sensitive process consisting of two different systems. The system associated with the luminal aspect of the cell appears to be dependent on oxidative metabolism, whereas the system associated with the contraluminal aspects of the cell appears to be independent thereof. Under several circumstances when the glomerular clearance of insulin falls thereby reducing the amount of insulin absorbed by the luminal aspect of the cell, contraluminal uptake increases, and a constant rate of insulin removal is maintained by the kidney.

## INTRODUCTION

The kidney is an important site of metabolism for several low molecular weight proteins (1). Insulin, a protein with the molecular weight of 6,000 is filtered at the glomerulus and then, like other low molecular weight proteins, it is reabsorbed by the proximal tubular cells (2–4). Reabsorption is of considerable magnitude as indicated by the finding that less than 1% of the filtered insulin is excreted in the urine in man (5, 6) and in the rat (7). In addition to being cleared from the circulation by glomerular filtration there is evidence, albeit indirect, that insulin is also removed from the peritubular vessels (5–7).

As there is little information about factors affecting the renal handling of insulin, we set out to determine (*a*) the relative contributions of glomerular and peritubular clearance to the renal clearance of insulin under different conditions, (*b*) what metabolic factors influence the ability of the tubular cells to remove insulin from the glomerular filtrate and the peritubular compartment, and (*c*) whether luminal and contraluminal uptake of insulin are influenced by the same factors.

The study was carried out with the isolated perfused rat kidney, as this model permits the use of maneuvers that cannot be performed in the intact animal. Furthermore, in the isolated kidney, events on the contraluminal side of the tubular cell may be exaggerated and thus, more readily studied. This is a consequence of a reduced glomerular filtration rate (GFR)[1] in the

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[1] *Abbreviations used in this paper:* GFR, glomerular filtration rate; NEM, *N*-ethylmaleimide; OCi, organ clearance of insulin.

presence of a perfusion flow which is severalfold greater than the renal blood flow in the intact rat.

The results of this study suggest that the luminal and contraluminal processes for insulin uptake may differ and that a reciprocal relationship between the two processes may exist in that contraluminal uptake increases when luminal uptake decreases secondary to a fall in GFR.

## METHODS

Male Sprague-Dawley rats weighing 290–410 g were used in all the experiments. Unless otherwise stated the rats were allowed free access to food and water. Perfusion of the rats' kidney was carried out according to the description of Nishiitsutsuji-Uwo et al. (8) as modified by Ross et al. (9). After inducing anesthesia with 60 mg/kg intraperitoneal pentobarbital, 50 mg/100 g mannitol and 200 U heparin were injected intravenously. The abdomen was opened, a PE 10 catheter was inserted into the right ureter and the right renal artery was cannulated *in situ* via the superior mesenteric artery. The renal vein was not cannulated. Renal ischemia was avoided by starting perfusion flow before cannulating the renal artery. The perfusion medium, with a 62-ml average volume, was oxygenated continuously with 95% $O_2$ and 5% $CO_2$, and recirculated with a pulsatile flow (Watson Marlow MHRE 3 Flow Inducer) at a pressure of 80–100 mm Hg distal to the tip of the cannula. Temperature was maintained at 37°C. Krebs-Henseleit bicarbonate buffer containing 6.7 g albumin/100 ml was used as the perfusion medium. The medium expressed in millimoles per liter contained: sodium, 143; potassium, 5; calcium, 4.25; magnesium, 1.2; bicarbonate, 18; chloride, 116; phosphate, 1.2; and sulfate, 1.2 at a ph of 7.40 when gassed with 5% $CO_2$/95% $O_2$. The medium was initially prepared as a 10% solution of albumin (Pentex bovine albumin fraction V, Miles Laboratories Inc., Miles Research Products, Elkhart, Ind.) which was dialyzed against several changes of cold Krebs-Henseleit bicarbonate buffer for 72 h and then diluted immediately before use (9). The diluted medium was filtered through a 0.45-$\mu$m millipore filter. 5 mM glucose was then added as the source of exogenous fuel. After the start of the perfusion [$^{14}$C]inulin (Amersham/Searle Corp. Arlington Heights, Ill.) was added to the reservoir, and a control perfusate sample was obtained. At 10 min, highly purified porcine insulin (lot 615-1082B-108-I, a gift of Dr. R. Chance, Eli Lilly and Co., Indianapolis, Ind.) was added which produced an average insulin concentration of 19±1.23 ng/ml in the perfusate sample taken 10 min later. The kidney was allowed to stabilize over a 15-min period after the start of the perfusion, and then 10-min urine collections and seven 1-ml midpoint perfusate samples were obtained. The urine samples were collected in albumin-coated tubes to avoid the loss of insulin by adherence to glass.

The following experimental protocols were carried out: (*a*) In six experiments, the kidneys were perfused throughout the study with the standard perfusion medium and served as controls. Four of these kidneys were studied at perfusate insulin concentrations of 23.7±2.1 ng/ml, and two kidneys at 7.1 and 4.7 ng/ml, respectively. Two-factor analysis of variance revealed no significant difference between these kidneys when perfused at high and low insulin levels and hence the data were pooled. (*b*) A separate group, consisting of four fasted animals, was studied after food had been withheld for 48 h. (*c*) In four experiments, the kidneys were perfused with a glucose-free medium. (*d*) In four experiments, 2 mM potassium cyanide was added to the perfusion medium to inhibit respiration. The addition was made at least 5 min before starting the urine collections. (*e*) In six experiments, 0.1–1 mM *N*-ethylmaleimide (NEM), an inhibitor of insulin degradation, was similarly added. (*f*) In four experiments, the kidneys were perfused with cold medium (10°C). This was achieved by circulating cold water through a glass jacket surrounding the oxygenator.

*Analytical methods.* Insulin was assayed in perfusate and urine samples by a modification (10) of the double-antibody method of Morgan and Lazarow (11). Bovine serum albumin was added to the standards and to the urine samples to provide the same protein concentration as in the perfusate samples. Standard curves for insulin in urine and for insulin in perfusate containing either 2 mM KCN or 1 mM NEM, paralleled the regular standard curves. The between-assay coefficient of variation was 11.9% and the within-assay coefficient of variation was 2.4%.

Sodium was measured with an IL 143 Flame Photometer (Instrumentation Laboratory Inc., Lexington, Mass.). [$^{14}$C]-Inulin was counted in a Nuclear Chicago Isocap 300 liquid scintillating counter.

*Calculations.* The urinary clearance of insulin and [$^{14}$C]-inulin was calculated from their respective urine, plasma concentration ratios, and urine flow by standard formulas. The clearance of [$^{14}$C]inulin was used as a measure of GFR. Fractional excretion of filtered insulin was calculated as urinary insulin clearance/GFR × 100 and fractional excretion of filtered sodium as sodium clearance/GFR × 100. Perfusion flow was determined from timed measured collections of the venous effluent. The organ clearance of insulin (OCi) (in microliters per minute) was calculated according to the method of Mortimore et al. (12), as $k = 2.3\ V/\Delta t\ \log\ (C_1 - C_\alpha)/(C_2 - C_\alpha)$, where $k$ = organ clearance, $V$ = volume of perfusion fluid, $C_1$ and $C_2$ = initial and final concentrations over the time interval $\Delta t$, and $C_\alpha$ = asymptote approached by $C$ after prolonged perfusion. As preliminary experiments with low concentrations of insulin indicated the eventual disappearance of insulin after prolonged perfusion, $C\alpha$ was assumed to be zero. Similar results were noted when 1 mM NEM was added to the perfusion medium. Use of the above formula was validated by finding that (*a*) the disappearance of insulin from the medium at 37°C was monoexponential (Figs. 1, 2) and (*b*) there was a highly significant correlation ($P < 0.001$; $r = 0.943$) between the clearance of [$^{14}$C]-inulin determined by this formula and by the standard urine clearance formula (427±66 vs. 440±71 $\mu$l/min). Peritubular clearance was calculated as being equal to the difference between GFR and OCi. As it is unlikely that insulin is freely filtered, this calculation which assumes that the glomerular clearance of insulin is equal to GFR would probably underestimate peritubular uptake.

The average of all the clearance periods in each experiment was taken as the value for that experiment. Results are expressed as the mean±SEM. The data was statistically analyzed with a one-way analysis of variance and if the $F$ ratio was significant, Duncan's multiple range test was used to make comparisons among all the means (13) ($P < 0.05$ was regarded as significant).

## RESULTS

To exclude the possibility that insulin was disappearing from the medium due to the release of enzymes or because of spontaneous degradation, perfusate samples were obtained after removing the kidney from the circuit in several experiments. In the absence of the kidney, there was essentially no change in insulin concentration (Fig. 1).

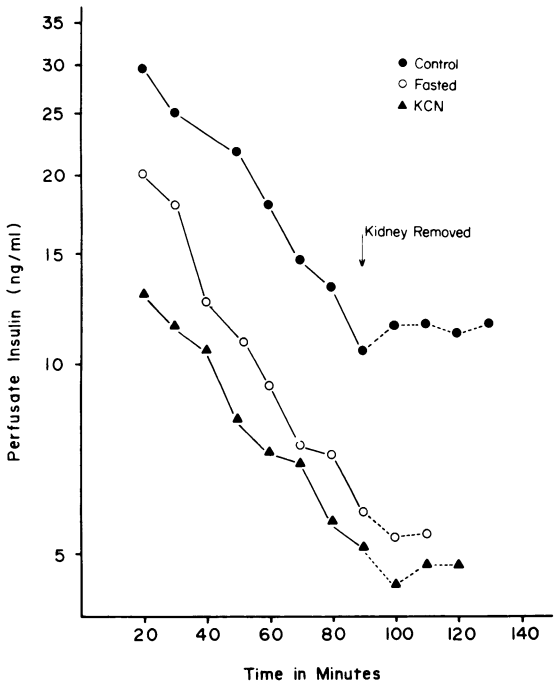


FIGURE 1 Effect of removing the kidney from the perfusion circuit on the disappearance of insulin from the perfusion medium. Kidneys from the control, fasted, and KCN-treated groups are shown.

*The OCi.* (Table I) The clearance of insulin by control kidneys averaged 974±63 μl/min. Fasting for 48 h was associated with an OCi of 984±60 μl/min, whereas in kidneys perfused with a glucose-free medium, the OCi was 1,013±43 μl/min. Both these values were close to that found in the controls. Addition of KCN did not significantly alter the OCi which averaged 970±92 μl/min, therefore indicating that insulin removal is not dependent on oxidative metabolism. Perfusion of kidneys with the sulfhydryl-group blocker, NEM, significantly depressed the OCi to 518±71 μl/min.

Cooling the kidney to 10°C greatly depressed the ability of the kidney to extract insulin (Fig. 2). OCi was not calculated in these experiments because there was little change in perfusate insulin concentrations. At this low temperature GFR fell to 167±10 μl/min and the fractional excretion of insulin rose to 69±3.3%, indicating that a small amount of insulin was being removed by glomerular filtration, a process dependent on mechanical forces.

*The contribution of glomerular and peritubular clearance to OCi.* (Table I). In control kidneys, GFR = 440±71 μl/min which was 46.3±8.2% of the OCi, and in kidneys of fasted animals, GFR = 419±54 μl/min which was 44.1±7.0% of the OCi, values which do not differ significantly from each other. As glomerular clearance could account for only part of the insulin removed, it is presumed that the balance was removed by contraluminal uptake from the peritubular compartment (peritubular clearance). In the absence of glucose, GFR fell to 251±35 μl/min, which was 24.8±3.2% of the OCi, whereas in the presence of KCN, GFR fell to 84±12 μl/min which was 8.6±1.1% of the OCi. In both these circumstances, despite a significant fall in GFR as compared to the controls, OCi remained essentially unchanged. There was no significant correlation between GFR and OCi, in control, fasted, glucose-free, and KCN-treated kidneys (Fig. 3). NEM produced a significant depression of OCi and GFR (518 ±71 and 75±12 μl/min) (Table I).

*The relationship between the GFR and the peritubular clearance of insulin is depicted in Fig. 4.* In control, fasted, glucose-free, and KCN-treated kidneys, groups between which OCi did not differ significantly, regression analysis showed a significant inverse corre-

TABLE I
*Function in Isolated Rat Kidneys Perfused Under Different Conditions**

| | Control | Fasted‡ | Glucose-free medium | KCN (2 mM) | NEM (0.1–1 mM) | Cold (10°C) |
|---|---|---|---|---|---|---|
| No. | 6 | 4 | 4 | 4 | 6 | 4 |
| OCi, *μl/min* | 974±63 | 984±60 | 1,013±43 | 970±92 | 518±71§ | —‖ |
| GFR, *μl/min* | 440±71 | 419±54 | 251±35¶ | 84±12¶ | 75±12¶ | 167±10¶ |
| GFR/OCi × 100 | 46.3±8.2 | 44.1±7.0 | 24.8±3.2 | 8.6±1.1§ | 16.3±3.2§ | — |
| FEi × 100 | 9.7±1.7 | 17.5±2.0 | 15.4±1.6 | 41.9±3.7§ | 50.6±6.2§ | 69.0±3.3§ |
| FEna × 100 | 2.6±0.5 | 8.6±0.8 | 20.7±3.2§ | 42.5±1.9§ | 80.0±3.8§ | 77.4±1.3§ |
| RPF, *ml/min* | 43.5±3.2 | 44.7±1.0 | 45.0±3.3 | 45.3±1.4 | 32.3±1.6§ | 28.0±0.9§ |

* Values are means±SEM.
‡ Kidneys from rats fasted for 48 h.
§ Significantly different when compared to controls using a probability level of <0.01.
‖ Not calculated—See Fig. 2.
¶ Significantly different when compared to control using a probability level of 0.05.
Abbreviations used in this table: FEi, fractional excretion of insulin; FEna, fractional excretion of sodium; RPF, renal perfusion flow.

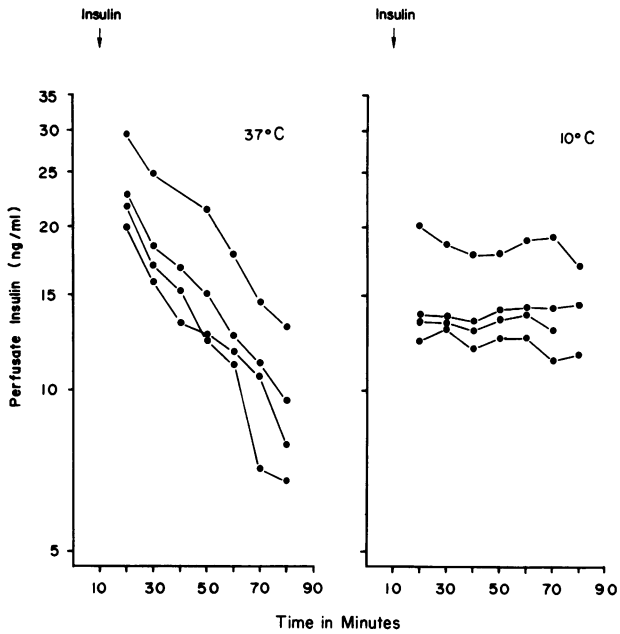


FIGURE 2 Effect of cooling the kidney to 10°C on the rate of insulin disappearance from the perfusion medium. Values from control kidneys perfused at 37°C are shown for comparison.

lation between GFR and peritubular clearance ($r = -0.828$, $P < 0.001$).

In the kidneys treated with NEM, peritubular clearance did not increase in proportion to the drop in glomerular clearance. Using stepwise discriminant analysis (14) with peritubular clearance and log (GFR) as variables, it was found that the NEM group was significantly different from all other groups ($P < 0.01$).

*Fractional excretion of filtered insulin and filtered sodium.* (Table I). In control kidneys the fractional excretion of filtered insulin and filtered sodium were 9.7±1.7 and 2.6±0.5%, respectively. Although fractional excretion of filtered insulin (17.5±2.0%) and filtered sodium (8.6±0.8%) was increased in the kidneys from fasted animals, these values were not significantly different from the controls. Similarly, in kidneys perfused without glucose, the fractional excretion of filtered insulin (15.4±1.6%), was not significantly different from that of the control kidneys, however the fractional excretion of filtered sodium (20.7±3.2%) was significantly greater than that of the controls. KCN significantly increased fractional excretion of filtered insulin (41.9±3.7%) and of sodium (42.5±1.9%). Addition of NEM also produced a significant increase in fractional excretion of filtered insulin (50.6±6.2%) and sodium (80±3.8%). In kidneys perfused at 10°C the fractional excretion of filtered insulin (69.0±3.3%) and sodium (77.4±1.3%) was significantly greater than in the controls.

*Renal perfusion flow.* (Table I). Perfusion flow in fasted (44.7±1 ml/min), glucose-free (45±3.3 ml/min), and KCN-treated (45.3±1.4 ml/min) kidneys were similar to that in the control kidneys (43.5±3.2 ml/min). In contrast, NEM and cold produced a significant fall in perfusion flow (32.3±1.6 and 28.0±0.9 ml/min).

## DISCUSSION

The kidney is a major site of insulin metabolism, removing insulin from the renal circulation in part by glomerular filtration (5–7). As filtration can at most account for 60% of the insulin removed by the human kidney (5, 6), it has been inferred that there must be a significant loss of insulin from the peritubular capillaries into the renal interstitium (5, 6). Insulin is then either taken up by the contraluminal aspect of the tubular cells, or alternatively, it is removed from the interstitium by lymphatic drainage (6, 15). In a recent study in the chicken (16), we noted that after injecting [$^{125}$I]iodoinsulin into the renal peritubular circulation, a large amount of the radiolabeled insulin left the peritubular vessels and accumulated in the kidney where degradation as measured by loss of immunoreactivity occurred, suggesting that lymphatic removal is not important. Strong support for this conclusion is obtained from the present study in which the lymph from the isolated kidney drained into the perfusion medium and could not therefore contribute to the dif-

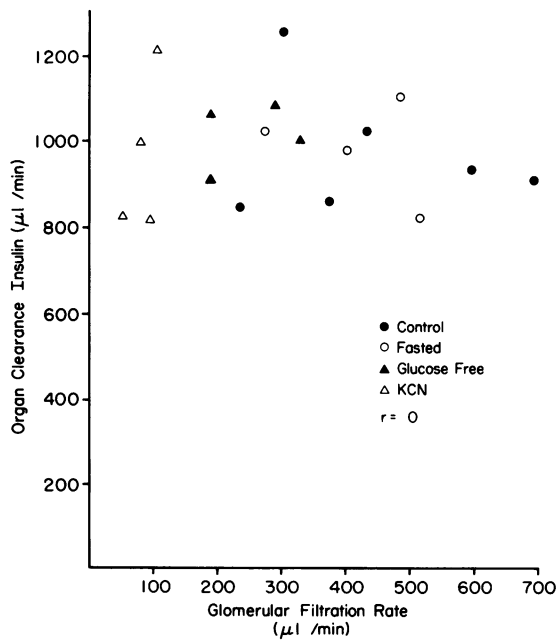


FIGURE 3 Relationship between GFR and OCi in control kidneys and in kidneys where the organ clearance does not differ significantly from the control values. This includes the fasted kidneys, and the kidneys perfused with a glucose-free medium, or a medium containing KCN. Individual kidneys are depicted and the different groups are represented by specific symbols.

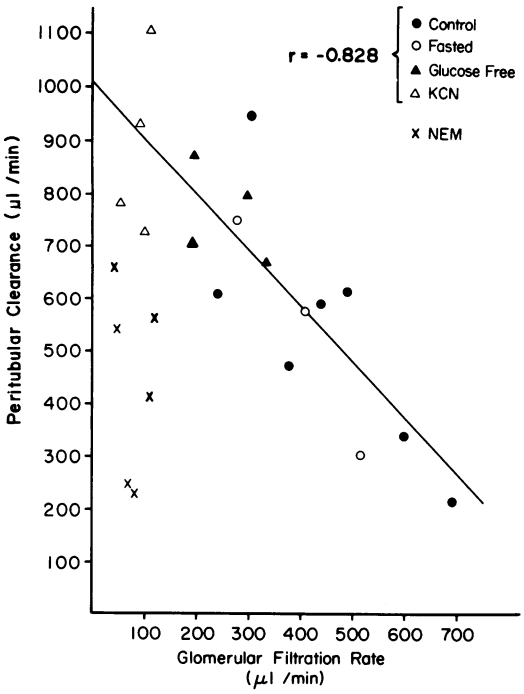


FIGURE 4 Relationship between GFR and peritubular clearance of insulin. The latter has been calculated as being equal to the difference between GFR and OCi. The calculated regression line, for values from control, fasted, glucose-free, and KCN-treated kidneys have been drawn. In these conditions, the OCi did not differ significantly from the controls. The values for NEM-treated kidneys have been inserted in the figure. Each kidney is depicted and the different groups are represented by specific symbols.

ference between the total amount of insulin removed by the kidney and the amount removed by filtration.

Although insulin, with a 6,000 mol wt, is not bound to protein (17) and at physiological plasma levels exists almost exclusively in its monomeric form (18), it is likely that some hindrance to its passage through the glomerular filtration barrier occurs. This would be the consequence of several factors including its shape, its negative charge, and its size (19–21). In the present study, when tubular reabsorption was depressed by cold (10°C), fractional excretion of filtered insulin rose to 69%, suggesting that the sieving coefficient of insulin (glomerular clearance of insulin/[$^{14}$C]inulin clearance) is at least 0.69. This figure may be different from the true sieving coefficient of insulin, because tubular function as judged by sodium reabsorption was not completely inhibited at 10°C and furthermore, glomerular permeability to proteins may be altered by cold. In a study of the glomerular clearance of porcine insulin carried out in dogs at plasma insulin levels above 0.3 U/ml, Franckson and Ooms (22) obtained a sieving coefficient of 0.59 for crystalline insulin. As this value was lower than might be expected for a protein the size of insulin, it was suggested that at the high plasma insulin levels employed, insulin circulated in the form of a hexamer. To what degree insulin is restricted by the glomerular filtration barrier at lower plasma insulin levels is not known, nevertheless, even at lower insulin levels, GFR would probably be an overestimate of the amount of insulin cleared by filtration. In the present study where the GFR of the control kidneys amounted to 46% of the OCi, peritubular clearance probably accounted for >54% of the insulin removed.

The importance of peritubular clearance in the isolated kidney is illustrated by the observation that GFR does not correlate with OCi. Furthermore, when GFR fell, peritubular uptake increased and the kidney maintained a constant rate of insulin removal (Fig. 4). It thus appears that the isolated kidney clears insulin from the circulation at a fairly constant rate, irrespective of whether the tubular cells take up insulin from the filtrate, or from the peritubular compartment. In this respect the isolated kidney handles insulin in a similar manner in which it handles arginine vasopressin, a small peptide hormone, with a mol wt of 1,084 (23). An alternative explanation for the constancy of the OCi at different GFR is that normally, very little insulin is filtered and that almost all of the hormone cleared by the kidney is removed from the peritubular vessels. Maneuvers which depress GFR but not the peritubular process would therefore not significantly affect OCi. However, for the reasons discussed before, it is likely that normally a large amount of insulin is filtered.

It is of interest to note that in the intact rat where the GFR is similar to renal clearance of insulin, Katz and Rubenstein (7) could only demonstrate peritubular clearance after reducing or abolishing glomerular filtration by ureteric ligation. Under these circumstances peritubular clearance was considerable, with a calculated arteriovenous insulin difference across the obstructed kidneys of 20%, which was approximately one-half of that found in normal unobstructed kidneys. It is apparent, therefore, that quantitative differences exist between the relative roles of the glomerular clearance and peritubular clearance of insulin in vivo and in vitro. These differences may be explained by the high perfusion flow and reduced GFR in the isolated kidney, a combination which would tend to exaggerate events on the peritubular side of the cells and reduce the role of glomerular filtration. The situation in vitro and in vivo also differs quantitatively in that fractional excretion of filtered insulin is 10% in the isolated kidney as compared to 0.5% in the intact rat (7). Despite these differences which necessitate caution in the extrapolation of data derived from the isolated kidney to the situation in the intact animal, the isolated kidney has been of great value in characterizing the mechanism whereby the kidney handles insulin.

In a recent study, Just and Habermann (24) demonstrated that several peptides, including insulin, bind

to isolated kidney brush border membrane and suggested that binding may be the initial step in endocytosis, a process that transports insulin from the tubular lumen into the cell (25), where degradation occurs. Although specific insulin binding receptors are present in crude kidney membrane preparations (26, 27), the exact location of these receptors has not been defined and their identity with the brush border insulin-binding process requires clarification. Endocytosis does not occur on the contraluminal aspect of the tubular cell, thus degradation of the hormone by the plasma membrane (28) possibly preceded by binding to specific receptors, (26–28) may be the process whereby insulin is removed from the peritubular compartment. It is also possible that the insulin removed may undergo a minor structural modification with loss of immunoreactivity but perhaps with retention of biological activity. Conceivably, insulin may also enter the tubular cell (15) by some process other than endocytosis (29). It may then undergo intracellular degradation as has been proposed for the liver (30, 31). Binding to receptors may also be the primary step in initiating the actions of insulin on the kidney, which includes sodium and phosphate retention (32, 33).

In a study on the metabolic requirements for protein uptake by the kidney, Miller et al. (34) found that the luminal uptake of horseradish peroxidase was abolished by cold and interference with oxidative metabolism. They also observed that luminal uptake did not require an exogenous supply of fuel. Interpretation of the study was limited as it was carried out using tissue slices incubated in a medium containing horseradish peroxidase. Studying the renal handling of lysozyme by a filtering isolated kidney, Maack (35) found that the metabolic inhibitors, KCN and iodoacetate, blocked the tubular absorption of the protein. In the present study, in which a filtering kidney was also used, we found that the luminal uptake of insulin was depressed by KCN and cold, and that unlike the situation for sodium reabsorption, exogenous glucose was not a requirement for insulin uptake. Essentially our results have confirmed those of the above mentioned workers. However, we have been able to extend our study to the contraluminal aspect of the cell and have observed that although contraluminal uptake is also sensitive to cold and does not require exogenous glucose as a fuel, KCN, in contrast to its effect on the luminal uptake of insulin, did not inhibit contraluminal uptake. To the contrary, although GFR fell to 9% of the OCi when KCN was added, peritubular clearance increased, and the OCi was maintained at control levels, suggesting that contraluminal uptake is not dependent on oxidative metabolism.

Of all the insulin removed by the isolated kidney only 5% was excreted in the urine. The remainder is presumably degraded by the active and specific insulin-degrading enzyme system (36, 37) present in the kidney and the resulting products of degradation, including perhaps biologically active material, are returned to the circulation (35). Fasting, a process which decreases hepatic insulin-degrading enzyme activity (38) did not alter the rate at which the kidney removes insulin. This does not indicate, however, whether or not the renal insulin-degrading enzyme activity was impaired, for delivery of insulin to its site of degradation may be the rate-limiting step in insulin removal. Cooling the kidney to 10°C depressed the rate of insulin removal from the perfusion medium, indicating that this is a temperature-sensitive process. This depressed rate of insulin removal may have been a consequence of several factors including (*a*) the depression of a temperature-sensitive process for carrying insulin to its site of degradation, as has been proposed to occur in the liver (39), (*b*) reduced binding of insulin to its receptors (41), (*c*) inhibition of insulin-degrading enzyme activity (39, 40) and (*d*) a reduced perfusion flow. It is unlikely that the reduction in perfusion flow was a major limiting factor, for although the flow was significantly lower than in the controls, it was still of considerable magnitude (28 ml/min). The OCi was also depressed by NEM. In addition to depressing the luminal uptake of insulin, NEM also inhibited the ability of the kidney to increase peritubular clearance in response to a falling GFR. It is not clear how NEM depressed the OCi, however, this may have been a consequence of inhibition of insulin-degrading enzyme activity (37) combined with an overall depression of renal function including perfusion flow.

The present investigation indicates that tubular cells remove insulin from the lumen of the nephron and from the peritubular compartment. Furthermore, our data suggest that insulin removal by tubular cells is a temperature-sensitive process, consisting of two different systems. The system associated with the luminal aspect of the cell appears to be dependent on oxidative metabolism whereas the other system associated with the contraluminal aspect of the cell appears to be independent of oxidative metabolism. Of particular interest is the hitherto unknown phenomenon observed in this study that under several circumstances, when GFR falls, thereby reducing the amount of insulin reabsorbed by the luminal aspect of the cell, contraluminal uptake increases, and a constant rate of insulin clearance is maintained by the isolated perfused kidney.

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