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Review article

The renal metabolism of insulin

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Summary. The kidney plays a pivotal role in the clearance and degradation of circulating insulin and is also an important site of insulin action. The kidney clears insulin via two distinct routes. The first route entails glomerular filtration and subsequent luminal reabsorption of insulin by proximal tubular cells by means of endocytosis. The second involves diffusion of insulin from peritubular capillaries and subsequent binding of insulin to the contraluminal membranes of tubular cells, especially those lining the distal half of the nephron. Insulin delivered to the latter sites stimulates several important processes, including reabsorption of sodium, phosphate, and glucose. In contrast, insulin delivered to proximal tubular cells is degraded to oligopeptides and amino-acids by one of two poorly delineated enzymatic pathways. One pathway probably involves the sequential action of insulin protease and either GIT or non-specific proteases; the other probably involves the sequential action of GIT and lysosomal proteases. The products of insulin degradation are reabsorbed into the peritubular capillaries, apparently via simple diffusion. Impairment of the renal clearance of insulin prolongs the half-life of circulating insulin by a number of mechanisms and often results in a decrease in the insulin requirement of diabetic patients. Much needs to be learned about these metabolic events at the subcellular level and how they are affected by disease states. Owing to the heterogeneity of cell types within the kidney and to their anatomical and functional polarity, investigation of these areas will be challenging indeed.

The kidney plays a central role in the clearance of insulin from the systemic circulation [1–4] (Fig. 1). Indeed, an estimated 30–80% of the insulin entering this compartment is removed by the kidney [4–7]. In normal man, approximately 40–50% of the insulin secreted by the pancreas is extracted during its first passage through the liver [2, 7]. Consequently, the kidney plays a smaller role in disposing of insulin secreted in normal man than in disposing of insulin injected into diabetic patients. Proinsulin, which is released from the pancreas in a lesser amount than insulin, also undergoes significant extraction by the liver [8], the kidney accounting for approximately 50% of the post-hepatic clearance of proinsulin [6]. In contrast, extraction by the kidney accounts for approximately 70% of the total rate of metabolic clearance of C-peptide [6], which undergoes negligible extraction by the liver [9]. Since in normal man C-peptide is secreted at the same rate as insulin [10], the systemic concentration of C-peptide may be used as a measure of pancreatic insulin release [11].

This review examines the physiological routes of renal insulin clearance; the interaction of insulin with renal insulin receptors; the enzymatic mechanism of renal insulin degradation; and, finally, the effect of renal insufficiency on insulin metabolism. As much remains to be learned about the renal metabolism of insulin, this review also attempts to indicate avenues of further exploration of these areas.

Physiological routes of renal insulin clearance: glomerular clearance

The kidney clears insulin from the systemic circulation by two anatomical routes: via the glomerular capillaries and the peritubular capillaries (Figs. 1 and 2). The major route is by way of glomerular filtration, which is followed by proximal tubular reabsorption and intracellular degradation of insulin. The rate of glomerular filtration of insulin and related peptides is determined by their size, shape, and charge; by glomerular permeability; and by effective renal plasma flow [12]. At physiological plasma concentration, insulin exists exclusively in its monomeric form (molecular weight 6000 daltons) [13] and is not bound to plasma proteins [14]. Nevertheless, insulin's properties seem to restrict its filtration through the glomerulus somewhat. Thus, the rate of glomerular filtration of insulin is approximately 90% that of inulin, a fructose polymer, which is filtered freely [15]. Less is known about the glomerular filtration of proinsulin and C-peptide, but most likely the rate of filtration of proinsulin is lower than that of insulin, while C-peptide probably is filtered freely.

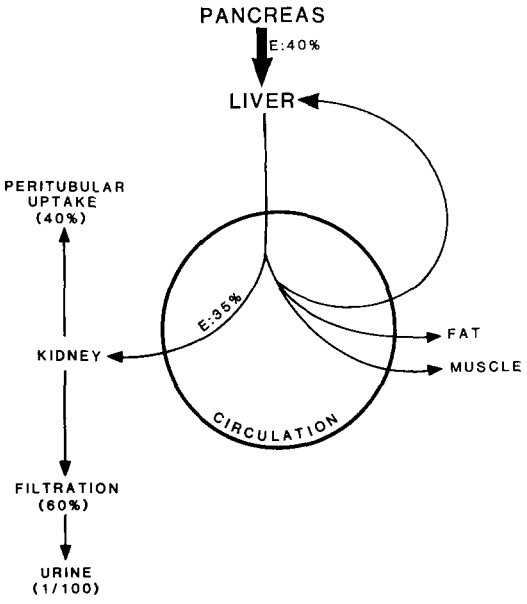

Fig. 1. Metabolism of insulin. E represents arterio-venous extraction [4]

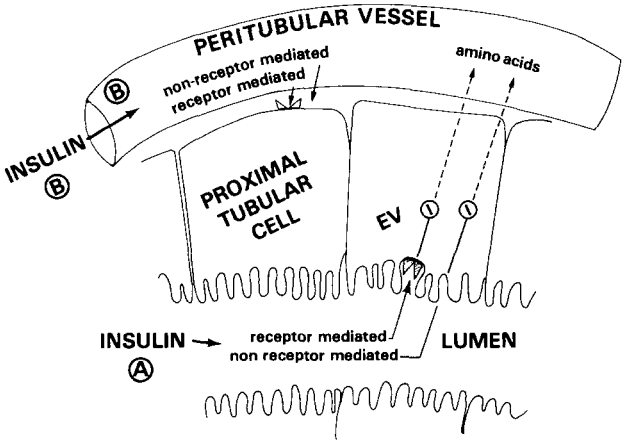

Fig. 2. Intra-renal pathways of insulin removal [3]. Both pathways involve both receptor and non-receptor mediated uptake. (A) Glomerular-filtration-tubular-uptake pathway. Filtered insulin is relatively resistant to brush-border enzymes and is internalized by endocytosis with some localization to lysosomes: The internalized insulin is degraded with the release of amino-acids into the peritubular vessels. (B) Insulin removed from the postglomerular peritubular vessels binds to the contraluminal cell membrane. I = insulin; EV = endocytotic vesicle; ⌒ = receptor

Although filtration of insulin through the glomerulus probably occurs non-specifically via simple diffusion, insulin may interact in a specific manner with glomerular cells, which contain insulin receptors [16, 17]. The biological consequences of this interaction are not known, in part because the cell-type bearing receptors has not yet been determined [16, 17]. As in other tissues, binding of insulin to glomerular receptors may influence glomerular metabolism [16] and, at least in isolated glomeruli, may lead to degradation of insulin. However, in vivo, the glomerulus is probably a trivial site of insulin degradation, so that insulin traverses the glomerulus intact.

Following passage through the glomerular filtration barrier, insulin enters the proximal tubular lumen, where normally more than 99% of the filtered insulin is reabsorbed by proximal tubular cells, with less than 1% appearing in the urine [1, 5, 6]. Autoradiographic studies indicate that luminal absorption of insulin occurs by means of endocytosis [18]. Evidently, luminal endocytosis may be initiated either by charge-mediated binding of insulin to non-specific brush-border-membrane sites [19], or, perhaps more importantly, by stereospecific binding of insulin to brush-border-membrane insulin receptors [20, 21]. In the isolated, perfused rat kidney, luminal uptake of insulin is impaired by low temperature; by n-ethylmaleimide and iodoacetate, thiol group alkylators that inhibit thiol enzymes, some of which degrade insulin; and by agents, such as KCN, ouabain, and chloroquine, which inhibit endocytosis in other tissues [22–24]. To some extent, the mechanism of luminal uptake of insulin is similar to that of other peptide hormones [3]. For instance, luminal uptake of insulin – like that of arginine vasopressin [25], growth hormone [26], and parathyroid hormone [27] – requires ongoing oxidative metabolism, and – like that of growth hormone – is inactive unless amino-acids are present in the luminal fluid [28]. Amino-acids are not needed simply as metabolic fuels, since a-amino-isobutyrate, a non-metabolizable amino-acid, supports luminal uptake of insulin. Rather, there seems to be some linkage of insulin uptake to amino-acid transport, although the nature of this linkage is unclear.

As a result of endocytosis, insulin is taken into proximal tubular cells within membrane-delimited endocytotic vesicles, in which it remains until these vesicles fuse with lysosomes [18]. There is no evidence of transtubular transport of intact insulin [15]. Rather, at some point, insulin is degraded to amino-acids, which diffuse across the contraluminal membrane eventually to enter peritubular capillaries [29]. It is presumed generally that insulin is degraded primarily in phagolysosomes. However, insulin-degrading enzymes are present at other subcellular locations [30–32], and several other peptide hormones, such as glucagon, angiotensin, and bradykinin, are degraded on contact with the enzyme-rich brush border membrane, and are absorbed, not in intact form, but as degradation products [33]. Unlike these small, linear peptides, insulin and other larger, more complex peptides are relatively resistant to the non-specific proteases of the brush-border-membrane, and, apparently, are absorbed in intact form. For example, the rate of insulin degradation by brush-border membranes is several-fold lower than that of glucagon degradation [34], and represents less than 2% of the total insulin-degrading activity of crude renal cortical homogenate [20, 21, 34]. It must be recognized, however, that such estimates may be inaccurate, because they are based upon assays, which detect degradation of insulin only after several cleavages of each insulin molecule [35]. Thus, it is possible that the degradation of insulin actually begins prior to fusion of endocytotic vesicles with lysosomes, with lysosomal proteases serving to convert early degradation products to amino-acids (vide infra).

Physiological routes of renal insulin clearance: peritubular clearance

In addition to luminal clearance via glomerular filtration, the kidney clears insulin from the post-glomerular, peritubular circulation (Fig. 2). In man, a little over one-third of the total renal clearance of insulin occurs via the peritubular route [5, 36]. Although less is known about peritubular clearance than about glomerular clearance, peritubular clearance is at least partly receptor-mediated [29]. Contraluminal insulin receptors have been identified in whole kidneys [29], in isolated tubules [37, 38], and in relatively pure isolated contraluminal plasma membranes [21]. These contraluminal receptors are not confined to the proximal tubule, but are distributed throughout the nephron, especially along the medullary thick ascending limb of Henle's loop and along the distal convoluted tubule. This distribution of contraluminal receptors is similar to the distribution of sites at which insulin is thought to affect tubular sodium reabsorption [38], suggesting that peritubular clearance of insulin may serve to deliver insulin to the sites of its major actions on the kidney (vide infra).

As an eventual consequence of removal from the peritubular circulation, insulin is degraded. However, the cellular site, timing, and mechanism of degradation are not yet known [29]. Although contraluminal uptake of insulin, like luminal uptake, is inhibited by low temperatures and by n-ethylmaleimide, unlike luminal uptake, contraluminal uptake is not affected by inhibitors of endocytosis [22, 23]. As expected from such observations, endocytosis is not a prominent feature of the contraluminal membrane of tubular cells, unlike the case at the brush-border membrane. In fact, there is no evidence that insulin, proteins, or other peptide hormones undergo endocytosis at the contraluminal membrane. This suggests that, via the peritubular route, insulin is degraded either at the contraluminal membrane itself or at an intracellular site, to which it is delivered by a process other than endocytosis [29].

Interaction of insulin with renal insulin receptors

The kidney clears insulin from the circulation by two different routes, which deliver insulin to two anatomically and functionally distinct membranes of the tubular cell. Early studies of a crude mixture of these plasma membranes detected the presence of insulin receptors [39, 40]. However, since a mixture of brush-border (luminal) and contraluminal membranes was used [39, 40], localization of the receptor population was not possible. Recently, insulin receptors have been identified in purified preparations of both contraluminal and luminal membranes [20, 21]. In both membrane preparations, there is also a large amount of non-saturable binding of insulin, suggesting that both membranes contain distinct specific and non-specific binding sites.

The binding properties of the insulin receptors in these isolated membrane preparations are similar to those of receptors in intact, isolated tubules [17, 38, 41] and in other tissues [21, 39]. The binding capacity of the contraluminal membrane is approximately four times that of the brush-border membrane, but the affinity of binding to the two membranes is similar. Unlabelled insulin at a concentration of 3 nmol/l displaces 50% of a monoiodoinsulin tracer. Proinsulin and certain analogues of insulin also displace monoiodoinsulin tracer, but less effectively than unlabelled insulin, while structurally unrelated hormones, such as glucagon, thyroid stimulating hormone, follicle stimulating hormone, and prolactin, do not displace monoiodoinsulin tracer at all. As in other tissues, Scatchard analysis of specific insulin binding yields a non-linear curve, suggesting that the affinity of the receptor population is heterogeneous, or that negatively cooperative site-site interaction of receptors occurs.

In extra-renal tissues, the binding of insulin to its receptor is followed by both biological response to and degradation of the hormone [2, 62]. Similar events also occur in renal tubular cells and, presumably, are receptor-mediated. In the kidney, one of the most significant actions of insulin is to increase sodium reabsorption [42, 43], apparently by stimulating Na-K-ATPase [44, 45]. Studies of isolated membrane preparations [45] suggest that this enzyme is stimulated by binding of insulin to contraluminal membrane receptors. Other major, direct actions of insulin on the kidney, such as stimulation of glucose and phosphate reabsorption, may also be mediated by binding of insulin to contraluminal membrane receptors [43, 46]. Thus, clearance of insulin via the peritubular route may be intimately linked to the expression of insulin's actions on the kidney. In contrast, there is little evidence that glomerular clearance is related to the renal actions of insulin. Rather, since the endocytotic activity of the brush-border membrane is greater than that of the contraluminal membrane, glomerular clearance is probably linked more intimately than peritubular clearance to the degradation of circulating insulin. For instance, during incubation of insulin with isolated tubules, a system in which clearance of insulin does not entail renal plasma flow, most of the insulin localizes within proximal tubular cells, where insulin-degrading activity is maximal [47]. This distribution is similar to that of luminal, but not contraluminal, membrane insulin receptors.

As noted above, degradation of insulin by the glomerular route probably involves luminal endocytosis of filtered insulin. Since luminal endocytosis may be mediated by either specific or non-specific binding of insulin to the luminal membrane [19–21], it is not clear whether insulin must bind to its receptor in order to be degraded. Degradation of insulin by isolated tubules can be prevented by pre-incubation of the tubules with trypsin, which inactivates insulin receptors, suggesting that degradation is receptor-mediated [37]. On the other hand, A_1–B_1 cross-linked insulin binds weakly to insulin receptors, yet inhibits ^{125}I-insulin degradation by isolated glomeruli or tubules more effectively than native

insulin [17]. Such conflicting results may have been influenced by variable release of degrading activity from tubular cells into the incubation medium, or by variable exposure of insulin to the luminal and contraluminal membranes of isolated tubules.

Enzymatic mechanisms of renal insulin degradation

As a result of clearance by the kidney, insulin ultimately is degraded. Degradation requires that insulin interact with the proximal tubular cell, either at the cell's luminal (brush-border) membrane or at its contraluminal membrane. Neither of these interactions is understood in detail. For example, the enzymes which catalyze insulin degradation, the subcellular sites at which they act, and the products which they form have yet to be identified. The principal renal insulin-degrading activity is inhibited almost completely by n-ethylmaleimide or para-chloromercuribenzoate [2, 31], suggesting that this activity resides in one or more thiol enzymes. This activity is also inhibited by bacitracin, which, in other tissues, inhibits the insulin-degrading enzymes, glutathione-insulin transhydrogenase (GIT) [48] and insulin protease [2]. Moreover, insulin-like growth factors, which are degraded by purified insulin protease [49] and inhibit its insulin-degrading activity [50–52], are degraded by renal cell membranes and cytosol [53] and inhibit insulin degradation by renal cell homogenate or subcellular fractions [53]. The effect on renal insulin degradation of other enzyme inhibitors, such as EDTA, L-1-tosylamide-7-amino-2-phenylethyl chloromethyl ketone, phenylmethanesulphonyl fluoride, and 1,10-phenanthroline, are variable, possibly because they do not act only on insulin-degrading enzymes [2].

Attempts have been made to define the subcellular distribution of renal insulin-degrading activity. Insulin-degrading activity has been observed in the cytosol, lysosomes, and mitochondria of proximal tubular cells [32]. Although a mitochondrial insulin-degrading protease has been isolated [54], the physiological significance of mitochondral insulin-degrading activity remains unclear. By contrast, other membrane-associated enzymes apparently act synergistically with cytosolic enzymes in degrading ^{125}I-iodoinsulin to trichloroacetic-acid-soluble products [30, 32]. This synergism suggests that cytosolic enzyme(s) catalyze an initial, 'specific' cleavage of insulin to trichloroacetic-acid-precipitable products, which are degraded to trichloroacetic-acid-soluble form by non-specific, membrane-associated proteases [55] that do not degrade intact insulin [31].

The predominant cytosolic insulin-degrading enzyme in the kidney is insulin protease [30, 56]. When a physiological concentration of insulin is used as substrate, this enzyme is the most active of all of the insulin-degrading enzymes in renal homogenate and subcellular fractions [31]. It hydrolyzes intact insulin to intermediate- and low-molecular-weight, non-immunoprecipitable, trichloroacetic-acid-soluble products via a series of disulphide-linked, insulin-sized, immunoprecipitable, trichloroacetic-acid-precipitable intermediates [56]. At least one of these 'insulin-like' intermediates, B_{16-17}-'clipped'-insulin, can be degraded to trichloroacetic-acid-soluble products by insulin protease itself, by glutathione-insulin transhydrogenase [57], or by non-specific proteases which do not degrade intact insulin [31, 55]. This synergism of cytosolic insulin protease and non-specific, membrane-associated proteases in degrading insulin suggests that insulin protease may catalyze the initial, 'specific' cleavage of insulin by kidney extracts.

Efforts to identify insulin-degrading enzymes in isolated renal cell plasma membranes indicate that, together, the luminal and contraluminal membranes contain only about 2% of the total insulin-degrading activity of renal homogenate [20, 21, 34]. Luminal membranes degrade insulin at twice the rate that the contraluminal membranes do, and contain several peptidases, including a neutral metalloendopeptidase which degrades the isolated B chain of insulin but not intact insulin [31, 55]. The isolated luminal membrane does not contain GIT activity [30] and does not produce intact A chains from insulin [20, 58]. Thus, as a result of interacting with luminal membranes, insulin may be degraded by the membrane-associated neutral metalloendopeptidase, which, in concert with insulin protease, degrades insulin synergistically to trichloroacetic-acid-soluble products [31, 55].

The insulin-degrading activity of the contraluminal membrane has not been characterized in detail. However, as observed with intact isolated renal tubules [17] and with isolated brush-border membranes [21], the degradation of ^{125}I-iodoinsulin by isolated contraluminal membranes is not saturable by a high concentration (10^{-6} mol/l) of native insulin [21], but, nevertheless, is inhibited more potently by native insulin than by unrelated peptides. This pattern of inhibition suggests that interaction of insulin with contraluminal insulin-degrading enzymes is at least partly receptor-mediated. As a result of this interaction, insulin may be degraded by insulin protease, but apparently not by GIT, since, as a result of peritubular clearance, insulin is converted to trichloroacetic-acid-soluble products via intermediates which are smaller than insulin, but different in size from the separate, but intact, A or B chains [29].

The contribution of these cytosolic and plasma-membrane-associated insulin-degrading enzymes to degradation of insulin by proximal tubular cells is not yet clear. As mentioned earlier, insulin is internalized by proximal tubular cells by means of endocytosis. As a result of endocytosis, most of which occurs at the luminal membrane, insulin is transported into cells inside of endocytotic vesicles, referred to as 'receptosomes' [59]. At least some of these receptosomes eventually fuse with various intracellular organelles that contain substantial insulin-degrading activity [18]. For example, the microsomal fraction of renal cells contains abundant GIT activity [60]. When a supraphysiological concentration of insulin is used as substrate, GIT may be the most active of all insulin-degrading enzymes in the kidney, although GIT activity may be lower than normal in the

kidneys of animals with chemically-induced diabetes. GIT, which is localized to the proximal tubule [61], reduces the disulphide bonds of insulin, producing separate, but intact, A and B chains, which can be degraded rapidly by the non-specific peptidases which are present in intracellular organelles [60], especially lysosomes. Together, GIT and lysosomal proteases could catalyze the initial, specific and later, non-specific steps, respectively, of insulin degradation by proximal tubular cells. However, several studies have presented evidence that GIT plays, at best, a minor role in renal insulin degradation [2, 30, 58].

As in other tissues, the properties and distribution of renal insulin-degrading enzymes suggest that proximal tubular cells may degrade insulin via either of two enzymatic pathways [2, 62]. One pathway may entail initial hydrolysis of insulin by insulin protease, followed by degradation of the resulting disulphide-linked intermediates by plasma-membrane-associated or lysosomal proteases, possibly in concert with GIT. This pathway apparently degrades insulin delivered to the proximal tubular cell by either glomerular clearance and luminal uptake or peritubular clearance and contraluminal uptake. The increased K_m of insulin degradation by the renal cell plasma membranes of diabetic rats suggests that this pathway may be less active in diabetes [63]. The abnormally low insulin protease activity in the tissues of diabetic rats supports this notion [64]. The recent demonstration that insulin protease is present on the exofacial surface of many types of cells [65] suggests that this pathway may also have earlier access to insulin than the other pathway does. The other pathway is thought to entail reductive cleavage of insulin by GIT, followed by hydrolysis of the separate but intact A and B chains by lysosomal proteases. This pathway would seem to require internalization of insulin, suggesting that it may degrade primarily that insulin which is delivered to the proximal tubular cell by glomerular filtration and luminal uptake. This pathway, like the enzymes which constitute it, may be most active when a supraphysiological concentration of insulin is presented to the proximal tubular cell [2].

It should be emphasized that the exact relationship between the two putative pathways of cellular insulin degradation is far from clear. Moreover, the relationship between each pathway and other events involved in the cellular processing of insulin (e.g., binding of insulin to its receptor, endocytotic internalization of insulin-receptor complexes, etc.) is not understood. Thus, the relative importance of these two enzymatic pathways to the two physiological routes of insulin clearance is not known.

Effects of renal insufficiency on insulin metabolism

In patients with advanced renal failure, basal plasma concentrations of insulin, proinsulin, and C-peptide are elevated [1, 66], and the relationship between the plasma concentration of insulin and that of C-peptide is abnormal [66]. The latter abnormality, which is due to greater renal clearance of C-peptide than of insulin, prevents use of plasma C-peptide concentration as an index of insulin secretion in patients with renal failure [66]. During the early phase of renal insufficiency, impaired renal insulin clearance is due to reduced renal blood flow, but as renal function declines, the effect of reduced renal blood flow is aggravated by a decline of tissue extraction of insulin, which falls from 0.4, the normal arteriovenous ratio, to 0.1 [5]. The relatively abrupt decrease in insulin requirement often seen in Type 1 diabetic patients as renal function deteriorates probably results from these decreases of renal blood flow and insulin extraction, together with the eventual uraemic depression of insulin degradation at extra-renal sites [67, 68]. A similar decrease of the rate of metabolic clearance of insulin is seen in non-diabetic patients with end-stage renal failure [69, 70]. In both diabetic and non-diabetic patients with renal failure, haemodialysis may accelerate metabolic clearance of insulin, suggesting that the lowering by uraemia of the metabolic clearance rate is due only in part to impaired renal insulin clearance [70, 71].

Animal studies confirm that renal failure suppresses insulin metabolism at several extra-renal sites. For example, insulin metabolism by muscle, which normally is a major site of systemic insulin clearance [67], is impaired in rats with acute or chronic renal failure [67, 68]. Similarly, insulin extraction by skeletal muscle isolated from acutely uraemic rats is depressed by two-thirds compared with normal muscle. In contrast, although the liver is also normally a major site of insulin clearance, neither acute nor chronic uraemia depresses insulin metabolism by the isolated, perfused, rat liver [67, 68]. However, the effects of uraemia on hepatic handling of insulin evidently are complex, since insulin binding to liver plasma membranes isolated from chemically uraemic rats has been impaired [68] in some studies, although normal [72] in others. Moreover, in hepatocytes isolated from uraemic rats, degradation is apparently suppressed, although receptor binding is increased [73]. Such findings are difficult to reconcile, but may reflect differences between studies in the duration or degree of uraemia or in the experimental procedures used, including that for iodinating insulin. Furthermore, factors producing short-lived, reversible effects on the binding and/or degradation of insulin may be present in the uraemic mileu but not in the in vitro study medium.

In insulin-dependent diabetic subjects, the consequence of impaired insulin metabolism in advanced renal failure is often a decrease in insulin requirement [15], and, in some patients with residual B-cell function, the need for exogenous insulin may disappear. In the occasional patient with end-stage renal disease managed by haemodialysis, hypoglycaemia may occur [15]. In addition to the prolonged persistence of circulating insulin, altered dietary and exercise patterns in uraemic individuals [74] may contribute to these phenomena. For additional information about the effects of uraemia on insulin handling in vivo [15, 75] or on insulin action in vitro [76, 77], the reader is referred to recent reviews of these subjects.

Under normal circumstances, the capacity of renal tubules to absorb filtered insulin is enormous and saturation does not occur; hence, urinary insulin clearance normally is constant over a wide range of plasma insulin concentration and is less than 1 ml/min [1]. When damage to the proximal tubules occurs, urinary insulin clearance increases [1, 5, 36]. Thus, urinary insulin clearance may be used as an index of proximal tubular function. Indeed, when pure glomerular damage produces proteinuria, as in some forms of the nephrotic syndrome, urinary insulin clearance is normal. In contrast, combined damage to glomeruli and tubules results in the appearance of high- and low-molecular-weight proteins, including insulin, in the urine. In poorly-controlled, non-ketotic [78] and mildly ketotic [79] diabetic children, urinary loss of insulin increases. In adults with early diabetes complicated by ketoacidosis, there is a reversible defect in tubular reabsorption of insulin, and this defect may persist even after metabolic control has been regained [80]. The several-hundred-fold increase in urinary insulin clearance seen in these circumstances does not affect insulin requirement, since the biological activity of filtered insulin is lost whether it is excreted in the urine or reabsorbed and degraded by the tubules. Although the exact mechanism of hyperinsulinuria in diabetic ketoacidosis is not known, it is probably due to a generalized defect in proximal tubular function, since the fractional excretion of β-2-microglobulin, and perhaps of growth hormone [81], is also increased in adults with this disorder.

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