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Renal filtration, transport, and metabolism of low-molecular-weight proteins: A review

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Low-molecular-weight proteins are a heterogenous group of substances which constitute a small but biologically important fraction of total circulating proteins. The group comprises enzymes (for example, ribonuclease, lysozyme), immunoproteins (for example, light chains of immunoglobulins, β_2 microglobulin), and peptide-hormones (for example, insulin, growth hormone, parathyroid hormone). Despite general recognition that the kidneys play an important role in the plasma turnover and in the maintenance of adequate plasma levels of small proteins, the study of the renal handling of these substances still constitutes one of the most underdeveloped areas of research in renal physiology. Nevertheless, recent advances in the field have been considerable and are the subject of the present review. Without attempting to be all inclusive, we will analyze the evidence regarding the extent, pathways, and mechanisms of the renal accumulation, filtration, absorption, and metabolism of small proteins. A final section will then deal with the renal turnover and the homeostatic role of the kidney in the maintenance of adequate plasma levels of these substances. A critical appraisal of the methods used to estimate glomerular sieving coefficients and renal turnover rates of small proteins will be given in some detail, because a correct determination of these parameters is essential to an understanding of the kidney's role in the disposal of circulating small proteins.

Some aspects of the renal handling of small proteins are common to all members of the group, for example, filtration, absorption, intracellular catabolism. Other aspects are probably specific for particular proteins, for example, interaction with receptors at the peritubular side. We will emphasize the former and only mention the latter briefly. The reader should be aware, however, that the heterogeneity of the group, both in molecular structure and biologic function, makes it likely that some proteins may be processed by the kidney by modes not encompassed in the generalized picture described in the present review.

I. Renal accumulation of administered small proteins

The initial suggestion that the kidneys play an important role in the disposal of circulating small proteins derives from data on the organ distribution of administered labeled or unlabeled enzymes and peptide hormones. Results of studies conducted from the early 50's to the present in intact animals and in the isolated perfused rat kidney with a host of small proteins (for example, insulin [INS], glucagon [GLU], ACTH, growth hormone [GH], PTH, follicle-stimulating hormone [FSH], luteinizing hormone [LH], ribonuclease [RNAase], and lysozyme [LZM]) demonstrate that 20 to 50% of the administered dose is distributed in the kidney [1-16]. The degree of renal uptake is related grosso modo to the molecular size of the protein. For example, in the flounder the renal volume of distribution ("space") of administered egg-white LZM (14,000 daltons) is at least two orders of magnitude greater than that of horseradish peroxidase (HP; 40,000 daltons) [7, 12, 17]. This inverse relationship between molecular size of the protein and degree of renal accumulation is to be expected if the process is primarily dependent on glomerular filtration of the protein.

The site of accumulation within kidney tissue has been shown by several investigators using autoradiographic and histochemical techniques to reside

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mainly, if not exclusively, in proximal tubular cells [8, 10, 13, 18–23]. As observed with larger proteins [24, 25], absorbed small proteins were mainly localized in endocytotic vesicles in the apical regions of the cell shortly after administration of the protein and in secondary lysosomes (phagolysosomes) thereafter [25–29]. In differential centrifugation studies on the localization of administered LZM and HP, part of the absorbed protein was found in the cytosol in an amount exceeding that expected from the disruption of lysosomes during the ultracentrifugation procedure [10, 21]. It is possible that the cytosolic localization of these proteins is the result of in vivo disruption of lysosomes caused by the concentration of a poorly hydrolyzable protein within these cell organelles. The subject of cytosolic localization of absorbed proteins, however, is controversial, and a detailed discussion of this point is beyond the scope of this article (see Refs. 10, 12, 28). Mitochondrial localization of administered ^{125}I-labeled bovine PTH has been reported [30]. By the time the animals were sacrificed after the administration of ^{125}I-labeled bovine PTH, however, it is probable that most of the hormone had already been hydrolyzed [16], and the autoradiographs most likely detected labeled catabolic products rather than the intact hormone.

The apical localization of protein in proximal tubular cells early after protein administration suggests that renal accumulation of small proteins proceeds via filtration and subsequent tubular uptake of the filtered proteins. However, accumulation via leakage from peritubular capillaries and subsequent uptake or absorption at the basal side of the cell has also been postulated. Definitive proof that the filtration-luminal absorption pathway is the major if not the exclusive route for renal uptake of small proteins has been obtained recently in experiments with ^{125}I-labeled rat LZM, rat GH, bovine PTH, and INS in the isolated perfused rat kidney [11–16]. Figure 1 summarizes the results of these experiments. When the proteins were added to the perfusate of a normal perfused kidney, extensive renal accumulation occurred, resulting in protein renal tissue to perfusate concentration ratios above 3.0 as compared to 0.3 for an extracellular volume marker. When tubular absorption of protein was inhibited by adding cyanide or iodoacetate to the perfusate (see tubular absorption section) while GFR and RPF were maintained at control levels, tissue accumulations of all four proteins fell dramatically to concentrations close to those of the extracellular volume marker. Similar dramatic decreases occurred for ^{125}I-labeled rat GH and bovine PTH when the perfused kidney was rendered a nonfiltering preparation, while RPF was maintained at control levels. The latter manuever was accomplished by raising the oncotic pressure of the perfusate to levels sufficient to counterbalance the filtration pressure. Under these conditions, GFR practically ceases, whereas the uptake of organic acids from the peritubular side remains unaffected [31]. These experiments provide definitive proof that the filtration-liminal uptake route is the predominant pathway for renal accumulation of the four proteins studied. They do not exclude, however, the possibility that some of these proteins may interact with receptors and be modified at peritubular sites (for example, INS, PTH, see section IV).

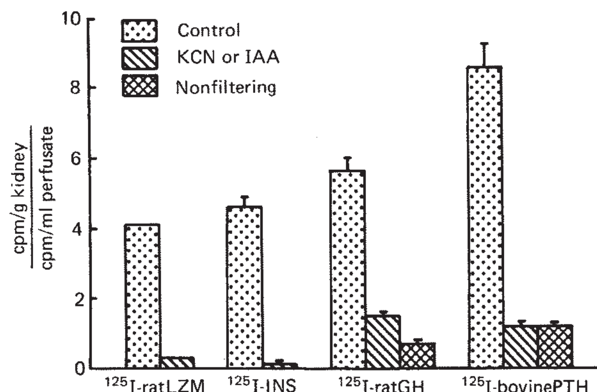

Fig. 1. *Tissue-to-perfusate ratio of radioactivity in isolated rat kidneys during perfusion with radioiodinated proteins, in control filtering kidneys, in kidneys perfused with iodoacetate (IAA, 10 mM) and in nonfiltering kidneys.* The GFR and renal perfusate flow (RPF) of IAA treated kidneys were not significantly different from that of control kidneys. In the nonfiltering kidney, GFR was negligible, and RPF was similar to that of the control filtering kidneys (see text). Tissue-to-perfusate ratio of ^{14}C-polyethylene glycol or ^{14}C-inulin in the nonfiltering kidney was about 0.3. Data is from Refs. 11, 12, 14–16, 39.

There has been justified caution in interpreting the results described above as proof or even indication that the kidney is an important organ for the disposal of endogenous circulating small proteins. First, in several of the experiments, heterologous or foreign proteins were used, and it is not certain that the disposal of these proteins is equivalent to that of endogenous proteins. Second, in several instances labeling of proteins with ^{131}I or ^{125}I may have resulted in denaturation, or loss of biological activity of the molecule, or both. This in turn could have altered the plasma turnover, or the renal handling of the labeled proteins, or both. Third, measurement of total radioactivity, or even trichloracetic-acid-precipitable radioactivity, in kid-

ney tissue after administration of labeled proteins does not necessarily differentiate between a labeled protein or labeled catabolites of the protein. Despite these limitations, there is strong evidence that studies on the renal accumulation of administered labeled proteins provide a useful indicator of the importance of the kidney in the disposal of their endogenous counterparts. This evidence can be summarized as follows:

(1) In the rat, renal accumulation of administered ^{125}I-labeled or unlabeled egg-white LZM does not differ markedly from that of labeled or unlabeled rat LZM [9, 11]. In the case of GH, renal accumulation of ^{125}I-labeled rat GH in the intact rat or in the isolated rat kidney was only slightly greater than that of ^{125}I-labeled human GH [13, 15]. Thus, at least for the proteins studied, the renal accumulation of heterologous forms do not differ greatly from that of their homologous counterparts.

(2) Denaturation and loss of biologic activity upon labeling with iodine is a serious problem, because it is extremely difficult to determine the biologic activity of the labeled molecules. However, mildly iodinated ^{125}I-labeled rat GH (less than 1 iodine atom per molecule protein), which retained its integrity, as attested by radioreceptor and radioimmunoassay, was accumulated extensively in kidney tissue [15]. Thus, it is likely that mildly iodinated proteins behave as the native molecule with regard to renal accumulation. Loss of biologic activity itself apparently does not affect the degree of renal uptake of a protein. Thus, the degree of accumulation of biologically inactive ^{125}I-labeled bovine PTH was found to be similar to that of a biologically active tritiated bovine PTH [8, 23]. It should be kept in mind, however, that strong iodination may lead to formation of labeled protein aggregates which, because of their large size, will result in a reduction in the renal uptake of labeled proteins [13, 15].

(3) Finally, in the cases of ^{125}I-labeled LZM, rat GH, and human GH, it was shown by chromatographic techniques that the label accumulated in kidney was attached to a molecule that eluted in the same region as the parent protein molecule [10, 13, 15]. Furthermore, the isolated perfused rat kidney accumulated these proteins to the same extent as did the intact rat, demonstrating that the radioactivity present in kidney could not have originated from catabolism of the protein in other organs with subsequent uptake of labeled catabolites by the kidney [11-16].

The data on the renal accumulation of administered proteins described so far indicates the importance of the filtration process as the initial step for the renal processing of small proteins. The quantitative aspects of the filtration of proteins will be considered in the next section.

II. Renal filtration of small proteins

Proteins are hindered at the glomerular filter in proportion to their molecular size, structure, and net charge. Factors which influence the filtration of macromolecules have been extensively reviewed before, and a full discussion of glomerular permselectivity is beyond the scope of the present article (see Refs. 32, 33). Unfortunately, the multifactorial influence of dimension (Stokes-Einstein radius) charges and the quaternary structure (asymmetry and rigidity of the molecular skeleton) is such that glomerular sieving curves obtained with artificial, charged or uncharged polymers and selected proteins cannot yet be used to estimate the glomerular sieving coefficient (GSC) of any one particular protein. Since the methods used to determine GSC's are difficult and sometimes marred by experimental and interpretative shortcomings, there are only a few small proteins for which the GSC is known with any degree of confidence. As will be seen later, however, the degree of filtration is the rate-limiting step for the plasma disappearance rate (metabolic clearance rate) of several small proteins. Therefore, a correct estimate of the GSC is of paramount importance to elucidate the mode and extent by which small circulating enzymes, immunoproteins, and protein-hormones are handled by the kidney.

Because direct measurement of small protein concentration in the glomerular filtrate has not yet been proven possible,[1] several indirect approaches

[1] Lack of reported direct measurements of concentration of small proteins in the filtrate may be surprising at a first view, particularly if one considers the relatively large number of reported determinations of the concentration of albumin and total proteins in the glomerular filtrate. The technical difficulties, however, are multiple: *(1)* enzymatic or biologic assays are not sensitive enough to detect any particular small protein in nanoliter samples of filtrate; *(2)* radioimmunoassays may be sensitive enough, but have the drawback, not only of cross reactivity between different protein species, but also, and most importantly, of the inability to discriminate between different forms of the same hormone, as is well illustrated in the case of PTH and GLU; *(3)* use of radioiodinated proteins may prove useful. Strong labeling procedures, however, which are used to obtain the requisite specific activities, may polymerize the protein molecule and give false values for its GSC. Even if polymerization could be avoided, it would be difficult to separate in glomerular filtrate samples the intact labeled molecule from labeled catabolites that may originate from the metabolism of protein in other organs; *(4)* finally, it is always difficult to solve the problems of binding of the protein to the glass of micropipettes used to sample the glomerular filtrate.

Table 1. Glomerular sieving coefficients of small proteins

Protein	Mol wt *daltons*	Stokes-Einstein radius[a] *Å*	Glomerular sieving coefficient
Insulin	6,000	(<16.4)	0.89 Method 2[b] isolated rat kidney [14]
Lysozyme	14,600	19.0	0.75 Method 1, dog [11, 36, 37]
			0.75 Method 2, isolated rat kidney [12, 39]
			0.75 Method 5, isolated rat kidney [12]
Myoglobin	16,900	18.8	0.75 Method 1, dog [51]
Bovine PTH	9,000	(~21.4)[c]	0.66 Method 2, isolated rat kidney [16]
			0.64 Method 3, isolated rat kidney [16]
			0.77 Method 3, dog [41][d]
Rat GH	20,000	(20.4)	0.72 Method 2, isolated rat kidney [15]
			0.58 Method 3, isolated rat kidney [15]
Horseradish peroxidases	40,000	31.8	0.007 (anionic) Method 5, rat [45]
		29.8	0.06 (neutral) Method 5, rat [45]
		30.0	0.34 (cationic) Method 5, rat [45]
Bence Jones (λ-L chain)	44,000	27.7	0.09 Method 1, dog [38]
			0.08 Method 4, mouse [43]

[a] Values were obtained or calculated (number in parenthesis) as in Ref. 31 (legend to Table 2, p. 188) except for horseradish peroxidases, which were obtained from Ref. 45.
[b] The indirect methods for obtaining the glomerular sieving coefficients are described fully in the text. The number in the table corresponds to the number of the method in the text.
[c] At plasma pH, bovine PTH behaves as an approximately 22,000-dalton globular protein (Ref. 46 and text).
[d] This was calculated from data given in Ref. 41 for kidneys with a normal GFR (see Ref. 16 and text).

have been used to estimate the GSC for small proteins. Table 1 summarizes values for the GSC of several small proteins obtained by different methods. A description and a critical appraisal of these methods follow below:

Method 1: Fractional clearance of proteins. The simplest of the indirect methods is the determination of the protein clearance (as a fraction of the GFR) at high plasma concentrations of the protein. The high plasma concentrations are necessary to minimize the decrease in urinary excretion rates due to tubular absorption of proteins. Unfortunately, the extent, as well as the kinetic characteristics, of the tubular absorption process is such that results obtained with this method must be interpreted cautiously. Thus, many authors have assumed that a linear relationship between urinary excretion and plasma concentration of a particular protein is indicative of saturation of tubular absorption [34-36]. A similar linear relationship is obtained, however, at high plasma concentrations of proteins if their fractional absorption is constant over a wide range of filtered loads, as was clearly shown to be the case for LZM [11, 12] (see section on "tubular absorption"). As a result, earlier determinations of the GSC of LZM [34, 36] gave values which were much lower (0.2 to 0.5) than the values obtained in more recent determinations (0.7 to 0.8) in which tubular absorptive capacity for LZM had indeed been exceeded [11, 37]. Studies with other proteins such as GH, PTH, and INS also provided evidence that linearity between urinary excretion and plasma concentration is not proof that the tubular maximum (T_m) for absorption of these proteins was exceeded (see section on "tubular absorption"). The high plasma concentrations of small proteins necessary to obtain reliable estimates of the GSC by the fractional clearance method may however introduce other artifacts. For example, GFR or permselectivity of the glomerular barrier may be altered by high plasma concentrations of proteins. A case in point is the observation that high plasma concentrations of egg-white LZM in the dog may produce, on some occasions, sudden falls in arterial pressure [36] (Maack, unpublished observations). Despite these drawbacks, the fractional clearance method, if judiciously used, may provide reliable values for the sieving coefficient of small proteins. Thus, the method provided estimates for the sieving coefficient of LZM of 0.7 to 0.8 [11, 37, 38] and for Bence Jones protein of 0.09 [39], values similar to those obtained by some of the methods described below.

Method 2: Fractional clearance of proteins in the absence of tubular absorption. The finding that tubular absorption of protein can be almost completely blocked by metabolic inhibitors such as cyanide or iodoacetate made it possible to determine the GSC of small proteins at normal plasma concentrations [11-16, 40]. In this approach, the fractional clearance of the small protein is measured in the presence of high concentrations of iodoacetate or cy-

Table 2. Fractional clearance and urine : perfusate ratios of small proteins in control isolated perfused rat kidneys and in isolated kidneys perfused with 10 mM iodoacetate or 3 mM cyanide[a, b]

Protein	Control			Experimental			Ref.
	$(U/P)_{GM}$	$(U/P)_{Pr}$	C_{Pr}/GFR	$(U/P)_{GM}$	$(U/P)_{Pr}$	C_{Pr}/GFR	
^{125}I-rat LZM	15.8	1.4	0.09	1.01	0.84	0.83	[11, 37]
Egg-white LZM			0.26 ± 0.05			0.77 ± 0.06	[12]
						0.08 ± 0.03	[12, 39]
^{125}I-INS	16.1 ± 1.2	1.2 ± 0.3	0.07 ± 0.02	0.98 ± 0.05	0.84 ± 0.05	0.86 ± 0.06	[14]
^{125}I-rat GH	23.8 ± 1.0	0.2 ± 0.05	0.02 ± 0.001	1.2 ± 0.1	0.91 ± 0.04	0.72 ± 0.03	[15]
^{125}I-bovine PTH	33.4 ± 0.3	0.50 ± 0.11	0.02 ± 0.001	1.2 ± 0.02	0.79 ± 0.03	0.66 ± 0.001	[16]

[a] For details of procedure consult the listed reference.
[b] Values are mean ± SEM of at least four experiments, except those for ^{125}I-rat LZM which are from a single experiment.
U/P is urine : perfusate ratio; GM is glomerular marker (creatinine, ^{14}C-inulin, or ^{14}C-polyethyleneglycol); Pr is protein.

anide. This method circumvents the influence of tubular absorption and thus avoids the drawbacks of method 1. Two limitations to this method, however, must be considered. First, it cannot be used in intact animals because of the high concentrations of inhibitors needed to abolish completely tubular absorption of proteins. Second, the inhibitors increase slightly (15 to 20%) but significantly the glomerular permeability to neutral dextrans of mean molecular weights of 20,000 daltons [15] and hence should not be used to determine the GSC of proteins larger than this size. Table 2 lists the GSC's of LZM, PTH, GH, and INS obtained by this method in an isolated perfused rat kidney preparation with near-normal function and normal glomerular permeability to neutral dextrans (for details of the preparation and function of the isolated perfused rat kidney, consult Refs. 15, 16, 41). The high value for the GSC of INS (0.9) obtained by this method is expected for a 6,000-dalton protein and is the only value thus far reported in the literature. The values for the GSC of LZM (0.7 to 0.8) and PTH (0.65) were in good agreement with those obtained with method 3. The GSC of rat GH (the larger of these proteins; mol wt, 20,000 daltons) was, however, about 20% larger than that determined by method 3 (see Table 1). A still greater discrepancy was observed when the GSC for a ^{125}I-labeled human GH preparation containing a mixture of 20,000-mol-wt and larger aggregates was measured by both methods (0.5 with method 2, 0.39 with method 3 [13, 15]). Therefore, it is apparent that method 2 is valid for the determination of the GSC of proteins smaller than 20,000 daltons but becomes less reliable as the size of the protein increases. This is not surprising, since it is to be expected that changes in glomerular permselectivity will affect predominantly the GSC of proteins with sizes closer to that of the filtration "pores" (see Section III).

Method 3: Fractional extraction method. In our view, this is the most reliable method for the indirect determination of the GSC of those small proteins which are extracted by the kidney solely by filtration. The method is based on the theoretical principle that for a protein which is extracted by filtration alone, the ratio of its renal extraction rate to the renal extraction rate of a glomerular marker (that is, the GFR) measures the concentration of the protein in the glomerular filtrate, relative to plasma. An independent proof that the protein is not appreciably extracted from the peritubular side (as compared to extraction by filtration) and that the protein is not reabsorbed intact from lumen to blood must be provided to validate the method. The GSC of ^{125}I-labeled rat GH and ^{125}I-labeled bovine PTH determined in the isolated perfused rat kidney by this method gave mean values of 0.58 and 0.64, respectively [15, 16]. From data of experiments reported in the literature in which renal extraction of immunoreactive bovine PTH and creatinine were simultaneously measured in the dog [42], it is possible to calculate that the GSC of bovine PTH in the dog was 0.77 ± 0.07, a value not significantly different from that of the GSC for ^{125}I-labeled bovine PTH (0.64 ± 0.07) in the isolated rat kidney [16]. Calculated values for the GSC of sheep GH in the sheep, with this method, were between 0.3 and 0.4 [43]. Because the administered sheep GH contained an unknown mixture of monomers and dimers of the hormone, it becomes difficult to interpret this result.

A great advantage of method 3 is that no extraneous experimental interventions, such as the use of inhibitors or high plasma protein concentrations, are needed. In principle, method 3 can be used to measure the GSC of small endogenous circulating proteins without the need of administering the protein to the experimental animal. Unfortunately, it

cannot be used for all small proteins since some of them (for example, INS) are probably extensively extracted from the peritubular side (see section V).

Method 4: Fractional renal catabolic rate. This method is a slightly more complex variant of method 3 and consists in determining the slope of the regression line obtained by plotting the plasma disappearance rate of a protein (measured as a fraction of the intravascular pool cleared from plasma per unit of time) against the clearance of creatinine (also as a fraction of the intravascular pool cleared from plasma per unit of time) [44]. The reader is referred to the discussion of Ref. 44 for a detailed description of this method. The GSC of λ-L chain Bence Jones protein (mol wt, 44,000 daltons) determined by this method in mice gave values between 0.07 and 0.09, in very good agreement with the GSC values obtained in the dog using method 1. Method 4 was also used to determine the sieving coefficient of LZM in humans and gave a mean value of 0.35 [45], which is lower than estimates of the GSC of LZM by methods 1 and 2 in other species.

The same limitations described for method 3 (that is, absence of significant peritubular extraction and absence of transtubular transport of intact protein) also apply to this variant. The disadvantage of method 4 is the usual large error in measurements of plasma-decay curves in intact animals. In our view, however, this variant is of great value when direct measurement of protein renal arteriovenous differences is not feasible or is very difficult as in small laboratory mammals or in humans.

Method 5: Renal tissue accumulation method. This is a relatively simple method for estimating the GSC of administered proteins that are accumulated by the renal tissue. The method was first used to test whether renal accumulation of lysozyme could be accounted for by filtration alone [11, 12]. In this method, the protein under study is infused in intact animals or is added to the perfusates of isolated kidneys. Plasma concentration and urinary excretion of the protein are measured in the course of the experiment together with the GFR. At the end of the experiment, the renal tissue content of the protein is measured. The GSC of the protein is given by the formula:

$$GSC_{Pr} = \frac{T_{Pr} + U_{Pr}V + DT_c}{GFR \times P_{Pr}} \quad (1)$$

where T_{Pr} is the amount of protein accumulated by the renal tissue by the end of the experiment; P_{Pr} is the plasma or perfusate protein concentration in the course of the experiment, $U_{Pr}V$ is the urinary excretion rate of the protein in the course of the experiment, and DT_c is the amount of filtered protein catabolized in renal tissue in the course of the experiment. For proteins which are slowly catabolized by renal tissue, such as LZM and HP, DT_c is negligible, and determination of their GSC becomes simple because T_c, $U_{Pr}V$, GFR, and P_{Pr} can be easily measured. Values of GSC of LZM obtained by this method in the isolated perfused rat kidney were not significantly different from those obtained with method 2 in the same preparation and with method 1 in the intact dog [12]. Very recently, this method has been successfully applied to a determination of the differences between the GSC's of anionic, cationic, and neutral horseradish peroxidases of the same molecular size [46].

When the renal catabolism of the absorbed protein in the course of the experiment is significant, as is the case with most peptide hormones, DT_c has to be determined independently. There is no available method to do this in intact animals. In the isolated perfused kidney, the renal catabolic rate of a protein can be determined by measuring the rate of efflux of catabolites from kidney tissue to the perfusate [15, 16]. The GSC of ^{125}I-labeled rat GH in the isolated rat kidney computed by this method (with a value for DT_c derived from results of efflux experiments) was 0.62, a value not significantly different from that obtained using method 3 (0.58; see above and Ref. 15).

The great advantage of method 3 is its simplicity, particularly when used for proteins which are catabolized very slowly by the kidney. Its main limitations are that it cannot be used for proteins that are taken up from the peritubular side, and steady state conditions must be assured (that is, P_{Pr}, $U_{Pr}V$, and GFR should remain relatively constant in the course of the experiment). Otherwise Equation 1 loses its validity.

All methods described above are indirect, and therefore the results obtained should be interpreted cautiously. As a rule, confidence in the reliability of the esimates for the GSC of a protein are greatly increased when independent determinations by two or more of these methods agree closely. Despite the limitations of the methods used to determine the GSC of protein, the following general conclusions regarding the filtration of small proteins can be drawn from the data obtained so far:

(1) Proteins smaller than 25,000 daltons (23 Å) cross the glomerular barrier, attaining concentrations in the glomerular filtrate that are usually more than 50% of their concentrations in plasma. Consid-

ering the magnitude of the GFR in mammals and the usually short plasma half-life of most small proteins, the filtration process accounts for a major portion of the removal of circulating small proteins from the vascular compartment (see Section V).

(2) Apparently, molecular dimensions and molecular shape (rigidity) are the main determinants of the degree of glomerular sieving of proteins smaller than 25,000 daltons or 23 Å. Below this size, net charge of the protein molecule has less of an effect than that observed for dextrans or proteins larger than 25 Å, such as albumin or horseradish peroxidase. Thus, the GSC of the anionic protein myoglobin (19 Å) is almost the same as the GSC of the similarly sized but highly cationic lysozyme (see Table 1). This finding does not contradict the critical importance of charge as a determinant of glomerular permselectivity of the larger proteins [33, 46]. It is reasonable to postulate that the closer the size of the protein molecule to the size of the "pores" of the glomerular filter, the larger will be the electrical hindrance for the passage of that protein to the filtrate. As previously postulated [32], the influence of symmetry and shape of the protein molecule on the GSC's of proteins is a major one. Thus, bovine PTH, a 9,000-dalton cationic protein, has only a slightly higher GSC than does rat GH, a 20,000-dalton anionic protein (Table 1). As previously pointed out [16], this apparent discrepancy can be explained by the high degree of asymmetry of the bovine PTH molecule, as discovered in studies of the elution characteristics of this protein on Sephadex [47]. Thus, when bovine PTH is eulted on Sephadex at a pH close to that of plasma, it migrates as a 20,000-dalton globular protein. At a very alkaline pH or in the presence of 6 M guanidine buffer (conditions which tend to randomize protein coils and hence decrease the degree of asymmetry of the molecule) it elutes as a 9,000-dalton globular protein. Therefore, it is not surprising that bovine PTH and rat GH have similar glomerular sieving coefficients [15, 16]. The influence of shape or rigidity of the molecular structure also has been postulated recently to explain the finding that horseradish peroxidases have a lower GSC than do dextrans of similar size and charge [46].

(3) The degree of sieving of a protein is not dependent on the biologic activity of the molecule itself. It is exclusively a function of the physical characteristics of the molecule (size, charge, shape) and those of the glomerular filter (GFR, permselectivity). Thus, the filtered load of a protein under normal conditions is directly related to its plasma concentration. As will be discussed in a later section (see Section V), this relationship is of fundamental importance to an understanding of the role of the kidney in the overall plasma turnover of small proteins.

III. Tubular absorption and urinary excretion of small proteins

The ability of the renal tubular epithelium, particularly that of the proximal tubular cells, to absorb proteins and colloids has been known since the first decades of this century [48, 51]. The process of renal cell uptake and catabolism of filtered proteins has fascinated cell biologists and renal pathologists ever since, and a variety of elegant morphologic studies have been performed to elucidate these phenomena [21, 22, 24–26].

For the purpose of this article, it suffices to summarize the major conclusions of these studies. It is generally accepted that proteins are absorbed by segregation into endocytotic vesicles at the apical borders of the tubular cells. Apparently, this is an exclusive property of the luminal cell membrane, because there is no evidence for the presence of an endocytotic apparatus on the basolateral membrane of tubular cells. The endocytotic vesicles migrate from the apical borders to the cell interior and eventually fuse with lysosomes. This as yet poorly understood process brings into contact the absorbed protein and the hydrolytic enzymes present in these cell organelles. After variable periods of time which may last from minutes to days, depending on the particular species of protein studied, the absorbed protein is no longer detectable within the secondary lysosomes. Results of intracellular localization studies are consistent with the hypothesis that reabsorbed proteins are catabolized within the renal cells.

The morphologic aspects of protein absorption were the same whether the protein was injected into the intact experimental animal or was infused directly into the lumen of perfused convoluted proximal tubules, in vivo or in vitro [25, 27, 29]. Differential centrifugation studies confirmed in general the morphologic findings described above [10, 22]. The endocytotic uptake and intracellular pathways of protein absorption were first described for larger foreign or heterologous proteins (albumin, hemoglobin, horseradish peroxidase), but in the last decade it was shown that small proteins such as GH, LZM, and INS are absorbed by the same process [10, 27–29].

Contrasted to the wealth of qualitative and morphologic studies, there is a relative paucity of quantitative studies regarding the tubular absorption and intracellular catabolism of filtered proteins. This is primarily due to the difficulties in determining the concentration of proteins in the glomerular filtrate, without which it is not possible to quantitate the absorption process.

A classical approach to the estimation of tubular absorption maximum (T_m) of a protein is to measure its urinary excretion rates over a wide range of plasma concentrations. For most proteins studied, above a certain plasma concentration there is a linear relationship between urinary excretion and plasma concentration. This is naturally similar to that observed for glucose and other small organic solutes at high plasma concentrations of these substances.

Based on this similarity, it has been postulated that the ordinate intercept of the regression line between plasma concentration (abcissa) and urinary excretion (ordinate) measures the maximum absorptive capacity (T_m) of that protein. The T_m of larger proteins determined in this manner was not significantly different from zero for some, such as Bence Jones protein (λ-L chain [33]) and free hemoglobin [35] but it had a significant albeit variable value for albumin [35]. The reliability of these results is, however, open to question. First, the fundamental assumption of this method is that a linear relationship between urinary excretion and plasma concentration of a protein demonstrates that the tubular absorption mechanism for that protein is saturated. As will be described below, depending on the range of plasma concentrations attained, it is, however, possible to demonstrate such a linear relationship while tubular absorption is not yet saturated. Second, for larger proteins with very low glomerular sieving coefficients, small changes in glomerular permselectivity or GFR during the course of the experiment may obscure or magnify the degree of tubular absorption of these proteins.

This indirect approach has been used also to estimate tubular absorption of smaller proteins, such as myoglobin (mol wt, 17,500 daltons) [52]. The estimated value for the T_m of myoglobin (approximately 3 mg/min) appears low, but it should be kept in mind that it is at least two orders of magnitude above the normal filtered load of any particular circulating endogenous protein smaller than 20,000 daltons.

True renal titration experiments (that is, experiments in which urinary excretion or tubular absorption are plotted against filtered loads rather than plasma concentration of the protein) were performed with egg-white LZM in the intact dog and in an isolated perfused rat kidney preparation [11, 12, 37, 38]. The difference between these and the above experiments is that the glomular sieving coefficient of LZM had been determined with a certain degree of confidence by several independent methods (see Section II). Results of LZM renal titration experiments in the dog revealed some previously unsuspected characteristics of the process of tubular absorption of proteins [11, 12, 37]. First, the T_m for absorption in the dog was about 1.0 to 1.5 mg/min, a value 50 to 100 times larger than the normal filtered load of endogenous lysozyme in the dog. Second, despite this large absorptive capacity, urinary excretion of LZM started at filtered loads close to normal; that is, there was a large difference between the threshold and the T_m of lysozyme. Third, within the large range of filtered loads between threshold and T_m, fractional absorption of LZM was relatively constant, amounting to about 50% of the filtered load of LZM. As a consequence, fractional LZM clearance (C_{LZM}/GFR) remained constant within this range. Therefore, increases in LZM filtered loads, above threshold and below T_m, led to a concomitant increase in the tubular absorption and urinary excretion of the protein. Similar results were obtained in the isolated perfused rat kidney, as shown in Fig. 2. Results of these experiments led to the conclusion that the LZM tubular absorption process is best described as a high capacity (as compared to normal filtered loads), low affinity transport process [11, 12, 38].

Studies with rat GH in the isolated rat kidney [15] and INS in the intact dog [53], or rat [54] also showed that the absorptive capacity remained unsaturated at filtered loads above normal for these proteins. Whether this characteristic applies to other small proteins as well remains to be determined.

In a few instances, tubular uptake of proteins was measured by microperfusion techniques. In the pioneer work [54], ^{131}I-labeled INS, RNAase, and albumin were microinfused into the tubular lumen of rats in vivo. Tubular absorption was determined by the difference between the amount infused and the amount of TCA insoluble radioactivity recovered in the urine. Iodothalamate was infused together with the proteins, and its recovery in urine was close to 100%. When proteins were infused at different points along the proximal tubule, tubular absorption varied depending on the site of infusion: 50 to 70% of the dose of ^{131}I-RNAase or ^{131}I-INS was absorbed

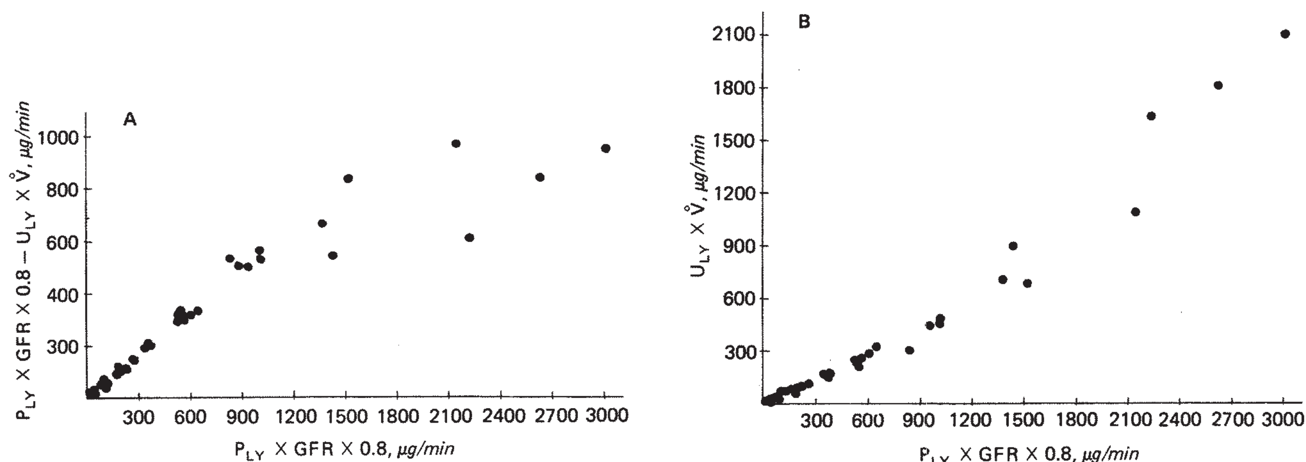

Fig. 2. *Renal titration of egg-white lysozyme in the isolated perfused rat kidney.* Lysozyme was added to the perfusate at the beginning of the perfusion and then between clearance periods. An equilibration time of 5 to 10 min was allowed between additions of lysozyme, and each clearance period lasted 10 to 15 min, perfusate samples being taken at midpoint. Lysozyme in perfusate (P_{LY}) and urine (U_{LY}) was measured as in Ref. 6. The results, partially reported before [12, 39] are from experiments in 21 isolated kidneys. Each *point* represents one clearance period, except at loads below 200 µg/min where several clearance points were combined for illustration purposes. 0.8 is the glomerular sieving coefficient of lysozyme [12, 39]. **A** *Tubular absorption of lysozyme, measured by the difference between filtered loads and urinary excretion rates of lysozyme (ordinate), is plotted against filtered load of lysozyme (abcissa).* **B** *Urinary excretion rate of lysozyme (ordinate) plotted against filtered loads of lysozyme (abcissa).*

when these proteins were infused in the early proximal tubule (first 20%). Absorption of proteins beyond the convoluted proximal tubules was either small or absent. Absorption of ^{131}I-albumin was less than that of ^{131}I-RNAase or ^{131}I-INS. Another interesting finding of these experiments was that as infused amounts of ^{131}I-INS were increased 400-fold, tubular absorption increased correspondingly, indicating a constant fractional absorption of this protein along the proximal tubule. Tubular absorption of ^{125}I-INS was also detected in experiments in isolated perfused convoluted tubules of the rabbit [27]. In the latter, the protein was added to the perfusion fluid, and tubular uptake was determined by measuring the radioacivity accumulated by the isolated nephron. Recently, the tubular absorption of ^{125}I-labeled human GH in isolated perfused proximal tubules of the rabbit was determined (Figueiredo and Maack, unpublished results). Figure 3 shows the results, and the legend gives a summary of the methodology used in this study. The results show that convoluted proximal tubules of the rabbit remove ^{125}I-labeled human GH from tubular lumen at a rate of about 50% of the load per millimeter of tubule length. In addition, Fig. 3 shows that there is a direct relationship between perfusion and absorption rates of the protein within the range of ^{125}I-labeled human GH loads tested. The results of all three studies described above show directly that the proximal tubule avidly removes protein from tubular fluid. In addition, the results are consistent with the conclusions, derived from the renal titration experiments with LZM, that the capacity of the absorption process is high compared to the estimated normal filtered loads of the small proteins studied.

The results obtained with small proteins do not necessarily conflict with the view held by some investigators that the T_m for albumin is close to the normal filtered load of this protein [55, 56]. Filtered loads of albumin (in terms of absolute amount of protein) are much larger than those of any particular small proteins or even of all small proteins combined. Therefore, it would not be surprising if the T_m for albumin is closer to its normal filtered load than that of LZM, GH, INS, PTH, or other small proteins. It should be pointed out, however, that due to uncertainties regarding the precise value of the albumin concentration in the glomerular filtrate, reported values for the T_m of albumin are not very reliable.

The question as to whether the uptake of proteins by proximal tubular cells is selective remains unsolved. Unfortunately, lack of adequate experimental data precludes all but a theoretical discussion of the subject. If proteins would be absorbed by a nonspecific endocytotic mechanism (that is, simple engulfment of tubular fluid containing the filtered protein), the tubular uptake of proteins would be a nonselective process. There is convincing evidence, however, that filtered proteins bind to the luminal

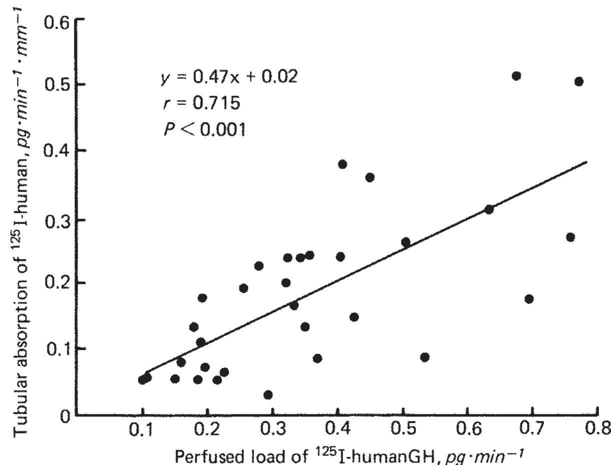

Fig. 3. *Tubular absorption of ^{125}I-labeled human GH by isolated proximal convoluted tubules of the rabbit.* Proximal convoluted tubules, dissected and perfused by the method described by Burg et al (*Am J Physiol* 210:1293, 1966) were perfused at 37° C with ultrafiltrate of rabbit serum to which ^{14}C-inulin and variable amounts of ^{125}I-labeled human GH (New England Nuclear Co.) were added. Perfusion rates were between 10 and 20 nl/min. All perfusion and collecting pipettes were siliconized. Control experiments showed that recovery of ^{125}I-labeled human GH from the pipettes used in the experiments was greater than 90%. TCA-insoluble radioactivity was measured in perfusion and collection fluids, and the concentration of labeled TCA-insoluble hormone was calculated from the specific activity of ^{125}I-labeled human GH. Tubular absorption rates were determined by the difference between perfused and collected amounts of TCA-insoluble radioactivity in each perfusion period. Six to eight perfusion periods were performed in each tubule, each period lasting from 8 to 10 min. In all experiments, perfusion with the commercial preparation of ^{125}I-labeled human GH led to an almost complete inhibition of fluid reabsorption by the proximal tubule. Whether this effect is due to impurities in the ^{125}I-labeled human GH preparation or to the hormone itself is unknown. Results shown are from eight tubules, each point representing one perfusion period. (Figueiredo and Maack T, unpublished results)

membranes (and/or the cell "coat") before segregation into endocytotic vesicles takes place [12]. As shown in Table 2, urine-to-plasma, or urine-to-filtrate ratios of LZM, GH, INS, and PTH are always much lower than those of markers of tubular fluid reabsorption [11-16]. Therefore, the concentration of these proteins in endocytotic vesicles must be greater than their concentration in tubular fluid, a phenomenon which would only occur if the protein binds to the luminal membrane or its "coat" before being incorporated into endocytotic vesicles. Despite this finding, is it very unlikely that there are specific receptors at the luminal membrane for each filtered protein or even group of proteins, which would provide a basis for their selective reabsorption. A certain degree of selectivity for uptake could still take place if the binding to the luminal membrane would be dependent on the charge, or the molecular size of the protein, or both. The finding that ^{131}I-albumin reabsorption by proximal tubules is less than that of ^{131}I-INS or ^{131}I-RNAase [54] and the recent observation that cationic amino acids increase urinary excretion of small proteins [57] provide some evidence for this hypothesis.

A related and also unresolved question is whether filtered proteins compete for the absorption process. Competition has been postulated on the basis of experiments that showed either a decrease in accumulation in renal cells or an increase in urinary excretion of a protein when filtered load of another protein is increased [58, 59]. Alternative interpretations, however, are possible. For example, in our hands [13], high concentrations of myoglobin led to an increased urinary excretion of LZM, in the isolated perfused rat kidney, but the phenomenon was not dose-related, a condition which is essential to prove competitive inhibition for absorption. The increased urinary excretion of LZM may have been the result of damage produced by high concentrations of myoglobin to the glomerular filter, or the tubular cells, or both. The same explanation could hold for the other instances in which large concentrations of any protein lead to increased urinary excretion or lack of renal accumulation of other proteins. The question of selectivity and competition for tubular uptake of proteins is clearly an open one, which demands much more investigation to be answered with confidence.

Tubular uptake of proteins is decreased in a dose-related manner by metabolic inhibitors such as cyanide and iodoacetate [11-16]. Table 2 shows the effect of high doses of these inhibitors on the fractional clearance of LZM, INS, GH, and PTH in the isolated perfused rat kidney. At high doses, the inhibitors completely block the tubular uptake of proteins, as shown by their negligible accumulation in kidney tissue (see Fig. 1). As expected, the high doses of iodoacetate or cyanide also strongly inhibit the reabsorption of sodium and water by the kidney [11-16]. Therefore, as previously pointed out [12], it is not possible to conclude from these experiments whether the effect of cyanide or iodoacetate on protein uptake by the kidney is direct or indirect. On one hand, it is known that iodoacetate or cyanide markedly affect endocytosis in other cell systems [60]. On the other hand, it is possible that the decrease in tubular uptake of proteins is a secondary result of the decrease in fluid and electrolyte transport. Evidence was obtained recently that seems to indicate that protein uptake is a specific process not directly dependent on net fluid and sodium reab-

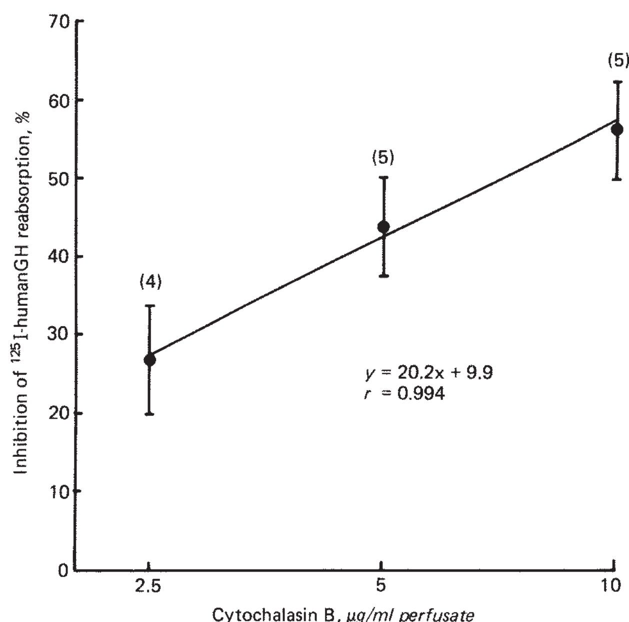

Fig. 4. *Effect of cytochalasin B on the tubular absorption of ^{125}I-labeled human GH by the isolated perfused rat kidney.* ^{125}I-human GH was added to the perfusate at the beginning of the perfusion, and two to three control clearance periods were performed. Then, cytochalasin B was added to the perfusate in the indicated concentrations, and after an equilibration period of 10 to 15 min, two to three additional clearance periods were performed. Tubular absorption of ^{125}I-human GH was calculated as in Refs. 13, 15. Results are expressed as percent inhibition of ^{125}I-human GH absorption, relative to control periods. Mean and SEM values for each dose are indicated in the figure. At the lower doses of cytochalasin B (2.5 and 5.0 μg/ml), the drug had minimal or no effect on GFR, sodium, water, or glucose reabsorption. At the highest dose these parameters were significantly depressed. (Data from Ref. 60)

sorption [61]. Thus, tubular uptake of a small protein (human GH) in the isolated perfused rat kidney remained unaffected as net sodium reabsorption was decreased by as much as 50% by lowering the oncotic pressure of the perfusate. In addition, and more importantly, cytochalasin B (a substance which affects microfilament function and endocytosis in other cell systems) inhibited the tubular uptake of human GH at doses which did not affect sodium, potasium, glucose, and water reabsorption by the isolated kidney. Results of these experiments with cytochalasin B are illustrated in Fig. 4. Therefore, these data suggest that specific inhibition of endocytosis decreases tubular absorption of proteins, without affecting other renal tubular functions.

Results of experiments in isolated perfused tubules of the rabbit indicate that some small filtered peptides, such an angiotensin and vasopressin, are hydrolyzed by brush border proteases [62]. It is doubtful, however, that these proteases are able to hydrolyze larger peptide-hormones such as PTH, INS, GH or other small proteins to any important extent. Indeed, inhibition of tubular absorption of labeled proteins by cyanide or iodoacetate leads to urinary excretion of TCA-insoluble radioactivity [11-16]. Because it is unlikely that these inhibitors affect brush border enzymes, this finding suggests that luminal proteases are unable to hydrolyze the protein to small fragments. Furthermore, combined autoradiographic and gel chromatographic studies with LZM and GH indicate that labeled proteins and not their labeled catabolites accumulate in renal cells, a result opposite to that expected if the protein had been extensively hydrolyzed by brush border proteases [10, 15]. Finally, even in the case of PTH where catabolites rather than intact protein are present in the cell at very early times after administration of ^{125}I-labeled bovine PTH, the evidence favors intracellular rather than brush border hydrolysis [16]. Despite this evidence, it cannot be completely ruled out that brush border proteases can modify the filtered proteins before their uptake into the renal cells.

Under normal conditions, only minimal amounts of small proteins are detected in urine. The studies described above show that this is due to extensive tubular absorption rather than to any sizeable hindrance to the passage of circulating small proteins into the glomerular filtrate.

Results of quantitative studies on the filtration and absorption of small proteins by several investigators have also provided the basis for an understanding of the pathophysiology of low-mol-wt proteinuria [11, 12, 38, 44, 63-70]. A brief summary of their conclusions follows. Increased urinary excretion of low-mol-wt proteins is primarily the result of either an increase in plasma concentration or a decrease in tubular absorption of small proteins. The former is observed in conditions in which there is an overproduction (or increased release to plasma) of specific small proteins, such as in multiple myeloma (Bence Jones proteins), mono- and myelomonocytic leukemias (lysozyme), and several other conditions too numerous to cite here. It should be emphasized that even small increments in plasma levels of small proteins may result in a significant urinary excretion, because, as pointed out above, a direct relationship between plasma concentration and urinary excretion of a small protein may be found at filtered loads below saturation of the absorption process. The other major condition for low-mol-wt proteinuria is impairment of tubular uptake of proteins by

tubular lesions, such as occurs in Fanconi syndrome, Wilson's syndrome, or chronic cadmium poisoning. Excretion of small proteins in these conditions is massive, up to 10,000-fold greater than in normal conditions. This is to be contrasted with the modest increase in urinary excretion of small proteins in lesions that affect primarily the glomerulus. The opposite relationship is found for larger proteins such as albumin, that is, modest albuminuria in primarily tubular lesions and massive albuminuria in primarily glomerular lesions. The contrast between the urinary excretion of small and large proteins in primarily glomerular, as opposed to primarily tubular, proteinurias is due on one hand to the differing glomerular permeabilities to these two groups and on the other hand, to the characteristics of the tubular absorption process of proteins. In primarily glomerular lesions, increases in glomerular permselectivity, and hence filtered loads of small proteins, is modest, because the glomerular sieving coefficient of these proteins is normally high. If tubular absorption is not impaired, part of the increased load of small proteins will be absorbed. Hence, any increase in urinary excretion will be minimized. That urinary excretion does increase at all in primarily glomerular nephropathies may be due to the generally low affinity of the tubular absorption process rather than to T_m's which are close to the normal filtered load. With larger proteins such as albumin, small changes in glomerular permeability will lead to large increases in filtered load. If the T_m of albumin is close to the normal filtered load, as postulated by some authors, the excess load will be entirely excreted into the urine. Even if the T_m of albumin is much larger than the normal filtered load, however, an increase in glomerular permeability will lead to massive albuminuria due to the low affinity of the absorption process. In primarily tubular nephropathies, on the other hand, the predominantly low-mol-wt proteinuria is due to impairment of tubular uptake of the normal proportionally high filtered loads of small proteins. Albuminuria will be less prominent than in glomerular nephropathies because of the proportionately lower filtered loads of albumin. The terms high- and low-molecular-weight proteinuria should be used, however, as relative terms, because, due to the very high plasma concentration of albumin compared to that of small proteins, albumin may still account for a large fraction of the total protein excretion in low-mol-wt proteinurias.

A rather unique aspect of the handling of small proteins by the kidney is that under normal conditions removal of filtered proteins from the circulation is accomplished mainly by tubular absorption and only minimally by urinary excretion. When tubular absorption is inhibited and urinary excretion correspondingly increased, however, the rate of removal of small proteins from plasma does not change significantly. Therefore, tubular absorption is not a rate-limiting process for the removal of small proteins from the circulation. This was first discovered in experiments which compared the plasma disappearance rate of Bence Jones proteins in normal mice and in mice in which tubular absorption was blocked by maleate [70]. Despite the large increase in urinary excretion in the latter condition, the plasma disappearance rate of Bence Jones protein was the same in both groups of animals. It thus became clear that absorbed Bence Jones protein must be catabolized by the renal tissue. The quantitative aspects of the renal catabolism of absorbed proteins will be considered next.

IV. Renal catabolism of small proteins

The rate of catabolism of an absorbed protein can be roughly determined by measuring its rate of disappearance from the tissue (intact kidney, kidney slices, or isolated perfused rat kidney) after the kidneys are preloaded with the protein in vivo or in vitro [7, 11-17, 28, 58, 71]. Figure 5 gives an example of the disposal of administered ^{125}I-labeled human GH from the intact rat kidney and from an isolated perfused rat kidney. Figure 5A shows that from 10 to 20 min after the administration of ^{125}I-labeled human GH, the concentration of radioactivity in kidney is maximal at a time when plasma concentration of TCA-insoluble radioactivity is already very low. Figure 5B, a chromatograph of kidney homogenate shows that the radioactivity accumulated in kidney tissue was eluted together with the labeled hormone; that is, absorbed ^{125}I-labeled human GH, and not a catabolite of the protein, was present in kidney tissue 20 min after administration of the hormone. During the next 20 min about 50 to 60% of the radioactivity disappeared from the kidney. Figure 5C shows the efflux of radioactivity from an isolated perfused rat kidney which had been preloaded with ^{125}I-labeled human GH in vivo. There is surprisingly good agreement between the rate of efflux of radioactivity from the perfused kidney (about 50% of the radioactivity initially present in the kidney was released to the perfusate in about 30 min) and the rate of decrease of radioactivity from the kidney of the intact rat shown in Fig. 5A. Figure 5D, a chromatograph of the

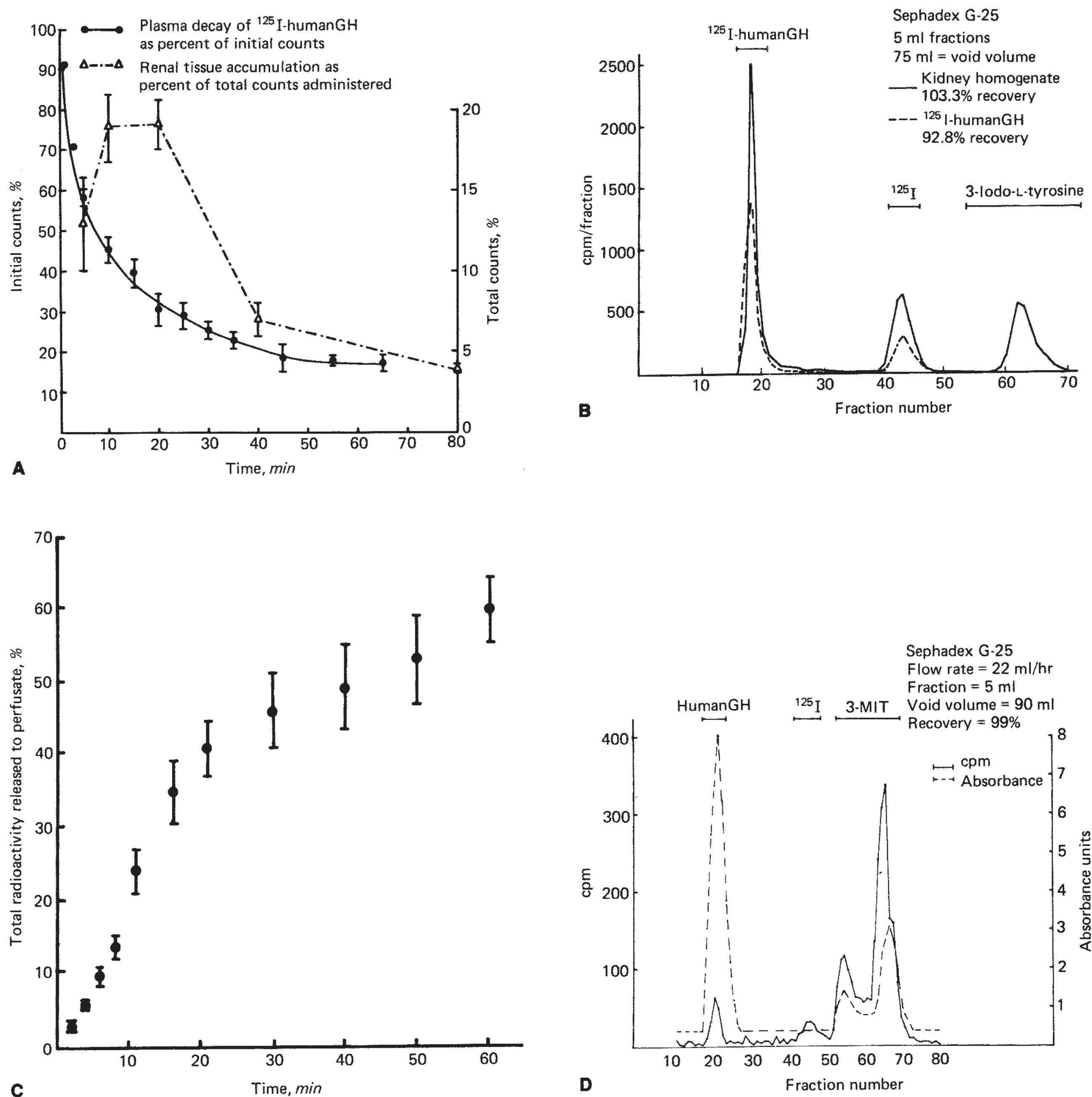

Fig. 5. *¹²⁵I-labeled human GH disposal by the intact and the isolated perfused rat kidney.* **A** *Plasma decay curve of ¹²⁵I-human GH (measured by the decay of TCA-insoluble radioactivity) and accumulation and disappearance of injected ¹²⁵I-human GH from kidneys of intact rats.* **B** *Elution pattern in Sephadex G-25 of radioactivity present in kidney homogenate 20 min after i.v. administration of ¹²⁵I-human GH to intact rats.* The elution pattern of the ¹²⁵I-human GH preparation used in this experiment is shown for comparison. The bulk of the radioactivity present in kidney homogenate was eluted in the same region as the intact labeled hormone. **C** *Efflux to perfusate of radioactivity initially accumulated in kidney tissue after the administration of ¹²⁵I-human GH.* Kidneys were loaded in vivo with the labeled hormone. At time of peak accumulation of radioactivity in kidney (10 to 20 min, Fig. 5a), the kidneys were removed from the animal and perfused in a closed-circuit system. The percent of total radioactivity initially present in the kidney released to the perfusate (*ordinate*) is plotted against perfusion time (*abcissa*). Values are mean ± SEM. Urinary excretion of radioactivity was less than 5% of that released to the perfusate. **D** *Elution pattern of radioactivity present in perfusate 60 min after perfusion of a kidney preloaded with ¹²⁵I-human GH in vivo.* The perfusate contained 10 mM unlabeled monoiodotyrosine to block the deiodination of ¹²⁵I-monoiodotyrosine by kidney deiodinases. More than 80% of radioactivity released from the kidney to the perfusate eluted in the same region as monoiodotyrosine (3-iodo-L-tyrosine). (Data from Ref. 13 and 15)

perfusate at the end of the experiment, shows that almost all of the radioactivity released to the perfusate is attached to a catabolite, ^{125}I-monoiodotyrosine. This finding, coupled with those from similar experiments with radioiodinated rat GH, bovine PTH, and egg-white LZM led to the conclusion that absorbed proteins are catabolized within the renal cells and that the products of catabolism are returned to the circulation [11, 16]. Efflux of radioactivity from kidney slices obtained from kidneys preloaded in vivo with radioiodinated egg-white LZM and cytochrome C gave results similar to those obtained in the isolated perfused rat kidney [28, 71]. The rate of catabolism of different proteins varied considerably, being of the order of minutes for most peptide-hormones. Among them, ^{125}I-labeled bovine PTH was the fastest (half-life in kidney tissue of less than 10 min [16]). For some enzymes the rate of hydrolysis is much slower, for example, absorbed egg-white LZM or rat LZM remains in kidney tissue for many hours [6, 9, 10–12], and horseradish peroxidase, for a few days [22]. More indirect evidence suggests that the rate of hydrolysis [72, 73] of light chains of immunoglobulins is also rather slow.

Regarding the products of hydrolysis of absorbed small proteins, the information available is rather limited. Because radioiodinated proteins were used in the type of experiments described above, only catabolites containing tyrosine could be detected. In the experiments with radioiodinated rat GH, human GH, LZM, and cytochrome C, in which breakdown by kidney deiodinases was obviated by the use of an excess of unlabeled monoiodotyrosine, the only detectable product of catabolism of these proteins was ^{125}I-monoiodotyrosine [12, 15, 28, 71]. In similar experiments with ^{125}I-labeled bovine PTH, an unidentified labeled catabolite was detected in the effluent of perfused rat kidneys. This unidentified product, however, may have been diiodotyrosine rather than a peptide containing tyrosine [16]. Since tyrosine is located in different positions within the molecules of the proteins tested, these data suggest that the catabolism of absorbed small proteins goes to completion, that is, the resulting catabolites are single amino-acids rather than peptides. It cannot be completely ruled out, however, that some small peptides originate from the intracellular hydrolysis of absorbed proteins and are returned to the circulation. In experiments with isolated perfused rat kidneys preloaded with unlabeled and labeled egg-white LZM, part of the absorbed protein was released intact back to the perfusate [12], confirming previous observations with this protein in flounder renal tubules [7]. Efflux experiments, however, in kidney slices from rats kidneys preloaded with ^{125}I-labeled egg-white LZM failed to detect the release of intact ^{125}I-LZM from kidney tissue [28]. Differences in the degree of iodination and dose of administered LZM may explain the discrepancy between these results. In our view, release of intact, absorbed LZM back to the perfusate does occur, at least in some conditions. In our experiments, it may have been the result of a large accumulation of this slowly hydrolyzable protein within lysosomes. This can lead to a disruption of lysosomal membranes, spillage of the protein together with lysosomal enzymes to the cytosol [10], and the eventual release of a part of the absorbed LZM to the perfusate. In addition, presence of absorbed LZM in renal tissue leads to a marked increase in the activity of some lysosomal enzymes [6]. This phenomenon may occur in those pathologic conditions where plasma levels of lysozyme (or other poorly hydrolyzable proteins, such as some of the light chains of immunoglobulins) are markedly elevated, as in monocytic leukemias or multiple myeloma. Contrary to the implications in our early studies with LZM [7, 10], however, release of intact absorbed proteins to the circulation is probably not the physiologic mode by which the kidney handles most filtered proteins [12, 15, 16].

The physiologic significance of the process of removal of small proteins from the circulation by renal catabolism rather than urinary excretion is unknown. As first pointed out in studies with light chains of immunoglobulins [44, 70], the rate of removal from plasma is the same regardless of whether the small proteins are absorbed and catabolized or are excreted in the urine (see last paragraph of section III). It could be argued that since the amino acids resulting from the intracellular catabolism of absorbed proteins are released back to the circulation, the organism conserves substances which are not important for the body economy. The amount of amino acids generated by the renal catabolism of small proteins, however, is insignificant compared to the total body pool of amino acids. A more provocative speculation is that the catabolic products resulting from the renal hydrolysis of a protein may exert some feedback control over the production or release of this protein to the circulation. Clear experimental evidence for this hypothesis, however, is lacking.

Partial hydrolysis of small proteins by the kidney has been reported only for PTH. Studies in the isolated perfused dog kidney show that intact immuno-

reactive bovine PTH (1–84 bovine PTH; mol wt, 9,000 daltons) is hydrolyzed to a 7,000-dalton fragment, which is said to be biologically inactive, and possibly to a 3,500-dalton fragment (bovine PTH 1–34), which is biologically active [74]. Apparently, this partial hydrolysis takes place at the peritubular side, but the 7,000-dalton fragment can only be disposed of by the kidney via a filtration-absorption route [75]. This observation may explain the finding of increased plasma levels of the 7,000-dalton PTH fragment in renal failure [74, 75]. Since PTH has its renal receptors at the peritubular membranes, it is not surprising that this protein may be partially hydrolyzed at peritubular sites. The partial hydrolysis of INS at peritubular sites could also explain the finding that the renal extraction of immunoreactive INS is greater than can be accounted for by filtration alone [76]. Whether other small circulating proteins can also be partially hydrolyzed via the peritubular route remains to be determined. In any event, the data obtained so far indicate that even if partial peritubular hydrolysis occurs, the final renal catabolism of a circulating protein takes place via the filtration-absorption route.

The renal catabolism of an absorbed protein is not rate-limiting for its plasma disappearance, except perhaps for proteins which are hydrolyzed very slowly by lysosomal enzymes. Indeed, if an absorbed protein cannot be returned intact to the circulation, it will eventually be hydrolyzed within the renal cells. In steady-state conditions, the rate of hydrolysis must be equal to the rate of tubular absorption; otherwise there would be an infinite increment in the renal accumulation of this protein. Therefore, the rate of hydrolysis cannot be limiting for the plasma disappearance of a small protein. The rate of intracellular digestion, however, will determine in part the steady-state concentration of a small absorbed protein in kidney tissue. Indeed, the slower the catabolic rate, as compared to the absorption rate, the larger must be the steady-state concentration of the protein in kidney tissue to permit a balance between cell uptake and intracellular hydrolysis of the protein. The physiologic consequence of this phenomenon is unknown. The predicted inverse relationship between renal catabolic rate and renal accumulation of filtered protein, however, explains the presence of protein-absorption droplets when protein filtered loads are increased either by an increase in glomerular permeability (for example, albumin in the nephrotic syndrome) or by an increase in plasma concentration of small, poorly hydrolyzable proteins (for example, lysozyme in monocytic leukemias, Bence Jones proteins in multiple myeloma). Eventually, the high concentration of these proteins in renal cells may cause tubular damage and further deterioration of renal function [73, 77].

V. Role of the kidney in the overall plasma turnover of small proteins: Extent and pathways of renal extraction of small proteins

The finding that the kidney filters, absorbs, and catabolizes circulating proteins does not by itself define the role of this organ in the plasma turnover of small proteins. The degree to which the kidney contributes to this phenomenon was inferred initially from results of experiments comparing plasma levels and/or plasma half-lives of endogenous or administered proteins in intact and anephric experimental animals or humans. For all small proteins tested to the present, plasma level, or plasma half-life, or both were markedly increased in bilaterally nephrectomized animals, humans or in renal failure. The pioneer work in this regard was the study comparing endogenous serum ribonuclease activity in control dogs and in dogs which had undergone bilateral nephrectomy or a ureteral-venous anastomosis [78]. It was then shown that serum RNAase activity increased markedly after bilateral nephrectomy, in direct proportion to the increase in serum creatinine concentrations. When the urine was returned to the circulation via the ureteral-venous anastomosis, however, serum RNAase activity remained close to normal, whereas serum creatinine concentration rose as in the bilaterally nephrectomized dogs. These results led to the conclusion that the kidney inactivates serum RNAase. Since this study, other investigators using a similar approach found that the kidney also inactivates endogenous LZM activity or GLU immunoreactivity [9, 79]. In addition to these findings, numerous studies have revealed that in nephrectomized animals there is an increase in plasma level and/or plasma half-life of a host of endogenous circulating small proteins, including RNAase, LZM, INS, GH, GLU, PTH, β_2 microglobulin, Bence Jones proteins, and fragments of immunoglobulins [9, 43–45, 70, 72, 76, 79–91]. The results of all these experiments indicate that the kidneys play an important role in the disposal of circulating small proteins, but they do not permit a precise estimate of the extent of this phenomenon. An increase in plasma levels of proteins in nephrectomized animals or humans may be partly due to an increase in production rather than a decrease in renal catabolism, as is certainly the case with

PTH and possibly also with LZM [82, 93]. Measurements of plasma disappearance rate in the presence and absence of the kidneys may circumvent the above problem but may introduce other artifacts. Thus, on one hand, in the absence of the kidneys other organs may compensate partially or totally for the lack of renal catabolism of the protein. On the other hand, changes in body fluid compartments or other secondary events associated with bilateral nephrectomy may lead to a decrease in the rate of removal of small proteins by other organs. In the former case, the role of the kidney in the determination of the plasma turnover of a protein would be underestimated, while in the latter case it would be overestimated.

The renal turnover of a protein can be precisely measured by determining its absolute renal extraction rate. In intact animals, this parameter is measured by the product of the renal arteriovenous concentration difference of the protein and the renal plasma flow. The fractional renal turnover rate is defined as the ratio of the renal turnover to the total plasma turnover of the protein. The latter is taken as the plasma disappearance rate (metabolic clearance rate) of the protein. Theoretically, the fractional renal turnover rate of a protein defines exactly the degree to which the kidney contributes to the overall plasma turnover of that protein. In practice, several limitations are to be considered. First, if the renal arteriovenous concentration difference of a protein is small, the error of measuring its renal turnover rate becomes very large because of the normally high renal plasma flow. Second, plasma protein decay curves are multiexponential and thus difficult to analyze in detail. Third, and most important, the interpretation of the particular measurement of a protein is not always clear. Thus, proteins may be determined by enzymatic, immunologic, or biologic assays, which measure properties of only a portion of the protein molecule. Therefore, on one hand, a protein may be considerably modified by the kidney and still retain its enzymatic, immunologic, or biologic activity. On the other hand, it may be only slightly altered and yet may lose one or more of these properties. A case in point is the heterogeneity of circulating PTH [74, 75, 92, 93]. Different molecular forms of the hormone, the intact active form and a large inactive fragment, probably formed by partial hydrolysis of intact PTH in the kidney react similarly to some antibodies. In addition, two biologically active forms (1–84 and 1–34 PTH) cannot be discriminated by some antibodies. GLU is another example of a hormone circulating in plasma in different molecular forms—a 3,500-dalton and a 9,500-dalton peptide [79]. As more specific antibodies against portions of the protein molecules are developed and as plasma fractionation procedures improve, many peptide hormones, thought initially to be homogeneous, become in reality a heterogeneous group of molecules. Therefore, to measure the renal extraction of a protein, it is necessary in principle to characterize the molecule fully both in renal arterial and in renal venous blood. Labeling of the protein molecule circumvents some of the difficulties described above but may introduce other artifacts, such as denaturation, polymerization, or inactivation of biologic activity of the protein. Despite the problems mentioned above, there is consensus among investigators in the field that the kidney accounts for a major, if not the major, fraction of the overall plasma turnover of several circulating enzymes, immunoproteins, and peptide hormones. Experimental measurement of the fractional turnover rate in either intact animals or the isolated perfused rat kidney of several small proteins, including INS, C-peptide, proinsulin, GLU, PTH, GH, LZM, shows that 40 to 80% of their total plasma turnover can be attributed to renal catabolism [9, 15, 16, 42, 43, 76, 79]. These experiments were done with labeled or unlabeled and homologous or heterologous proteins. Thus, there is little doubt that the kidney is a central organ for the disposal of circulating small proteins.

The findings that the glomerular sieving coefficient of small proteins is generally high and that transtubular transport of intact protein does not occur for most proteins suggest that the filtration route is of primary importance for the renal extraction of small proteins. In addition, the absence of an endocytotic apparatus on the contraluminal side of tubular cells and the lack of evidence that circulating proteins are taken up into the cell from the contraluminal side suggest that renal extraction by peritubular uptake of proteins into the cell does not occur. As already pointed out, these data do not exclude the possibility that proteins may be inactivated or modified by interactions at the peritubular side, particularly if there are specific receptors for a protein on the basal cell membrane. The strongest evidence indicating the existence of a peritubular route for extraction of proteins comes from experiments with immunoreactive insulin. In several experiments in rats, dogs and humans, it was found that the renal extraction rate of this protein was larger than could be accounted for by filtration alone [76, 91, 94, 95]. Therefore, immunoreactive insulin must

have been removed from the renal circulation from the peritubular side in addition to its removal by filtration. There is, however, excellent evidence that at least labeled insulin is not absorbed into the cell from the peritubular side [27]. In addition, lymph flow in the kidney is too small to account for peritubular removal of insulin via the lymphatic route. Therefore, it is likely that proteases located at the peritubular side of the renal cells are able to at least modify the immunoreactivity of the INS molecule. For most proteins, however, renal extraction is less than the GFR, and thus there is no need to postulate the presence of a peritubular mechanism to inactivate circulating proteins. Peritubular extraction of several small proteins has been postulated, however, on the basis of experiments showing persistence of renal extraction in ureter-ligated animals [71, 75, 76, 79]. In these experiments, significant renal extraction or decrease in plasma disappearance rates which were less than those observed in nephrectomized animals were taken as evidence for peritubular extraction of the proteins studied. Fluid filtration rate, however, can still be considerable when ureters are acutely ligated. In addition, a considerable amount of protein can diffuse into the filtrate when GFR is very low. Therefore, it becomes difficult to quantitate the residual extraction of protein by filtration in ureter-ligated animals (see Ref. 89). Recently, a new experimental approach was devised to measure with more certainty the contribution of the peritubular side to the renal extraction of a protein [15, 16]. The method consists of measuring the renal extraction of a protein in an isolated perfused rat kidney in which filtration is abolished by raising the oncotic pressure of the perfusate sufficiently to counterbalance filtration forces. In this manner, renal perfusate flow and hence the delivery of the protein to the peritubular side is kept at the same levels as in the filtering isolated kidney. The integrity of normal peritubular uptake processes is maintained, as shown by the finding that accumulation of organic acids in the nonfiltering kidney does not differ significantly from that in the filtering kidney [31]. Renal extraction of proteins in both the filtering and nonfiltering isolated perfused rat kidneys can be easily measured by determining the perfusate disappearance rate of the protein [15, 16]. With this approach, it was found that peritubular extraction of homologous ^{125}I-labeled rat GH and heterologous ^{125}I-labeled bovine PTH was negligible compared to their extraction by filtration. In addition, it was found that extraction by filtration accounted for more than 60% of the plasma disappearance of the labeled hormones in the intact rat [15, 16]. The ^{125}I-labeled rat GH used in these experiments remained intact as far as could be assessed by radioimmunoassay and radioreceptor assay, and had the same plasma disappearance rate as unlabeled rat GH. The ^{125}I-labeled bovine PTH used, in addition to being heterologous, however, was probably rendered biologically inactive by the labeling procedure. It is noteworthy, however, that the fractional renal turnover of ^{125}I-labeled bovine PTH was similar to that determined for immunoreactive bovine PTH in the dog [16, 42]. The evidence obtained with GH and PTH in the isolated perfused rat kidney experiments does not rule out the possibility that these hormones are modified at peritubular sites, a modification which may be important for their biologic activity, as proposed for PTH [75]. It does prove, however, that the final extraction occurs essentially by a process of filtration and tubular uptake of the hormones. It should be emphasized that renal extraction by filtration is independent of the biologic activity of the protein and takes place whether or not the kidney is a target organ for a particular small protein. As previously pointed out [16], the nonspecificity of this process should not be taken to indicate that the renal extraction of small proteins by filtration does not have a biologic function or a homeostatic value. Essentially what this renal process accomplishes is the maintenance of adequate plasma levels of small proteins, because, under normal conditions, the renal turnover rate of a particular small protein is directly proportional to its plasma concentration. In this sense, the renal handling of small proteins is just one more aspect of the primary function of the kidney in mammals, the maintenance of balance in the internal environment.

Summary

Circulating small proteins are extensively filtered by the kidney. Included among these proteins are important peptide-hormones (for example, INS, GLU, PTH, GH), enzymes (for example, LZM, RNAase), and immunoproteins (for example, β_2 microglobulin, light chains of immunoglobulins). The glomerular sieving coefficient of circulating proteins, smaller than 20,000 daltons, is greater than 0.5, leading to filtered loads which are many fold larger than the plasma pool of these proteins.

Filtered proteins are extensively absorbed by the tubular epithelium, minimal amounts appearing in the urine. The tubular uptake process is characterized by a high capacity as compared to the normal

filtered loads of small proteins; it is directly or indirectly dependent on energy input, and it is inhibited by cytochalasin B, a substance which interferes with endocytosis in other cell systems. The mechanism of uptake can be best described as a specific endocytotic process because it depends on the binding of the protein to brush border membranes before the protein is incorporated into the endocytotic vesicles. The characteristics and extent of tubular absorption of small proteins explains the massive urinary excretion of small proteins when their plasma levels are elevated or when the tubules are damaged.

Absorbed proteins are hydrolyzed to amino acids within the renal cells, and these catabolic products are then returned to the circulation. The rate of hydrolysis depends on the nature of the protein, being of the order of minutes for most peptide hormones and many hours or days for enzymes and foreign proteins. The differences between the rate of renal catabolism among absorbed proteins is probably related to their degree of resistance to lysosomal acid hydrolases. Increased filtered loads of poorly hydrolyzable protein cause their accumulation in renal cells as "absorption droplets." This accumulation may lead to tubular damage and consequently to an increase in the urinary excretion of several low-molecular-weight proteins.

Although final catabolism of a circulating small protein occurs via filtration and tubular uptake from the luminal side, partial hydrolysis of some small proteins may take place at peritubular sites. This phenomenon may be of importance for the activation or inactivation of peptide hormones such as INS, PTH and GLU, but the physiologic role of this process is still unknown.

The kidney plays a major role in the disposal of several circulating small proteins, with renal extraction accounting for 30 to 80% of their plasma disappearance rates (metabolic clearance rate). The rate-limiting step for the removal of small proteins from the circulation is the rate by which these proteins are filtered by the kidney. The process of filtration of small proteins is obligatory in nature and homeostatic in result; obligatory in the sense that it does not depend on the biologic function of the protein but on the physical characteristics of the molecule (size, symmetry, charge, binding to larger molecules) and of the glomerular filter (GFR, permselectivity). The process of filtration is homeostatic in result because filtered loads—hence, renal catabolic, or urinary excretion rates, or both—are directly proportional to the plasma concentrations of small proteins. The increase in plasma concentrations of several small proteins in acute renal failure illustrates well the importance of the process of filtration for the disposal of circulating small proteins. The abnormal plasma levels of small proteins in conditions of marked decrease in GFR may contribute importantly to several of the symptoms observed in uremic patients.

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References

1. Sonenberg M, Money WL, Dorans JF, Lucas V, Bourque L: The distribution of radioactivity in the tissues of the rat after the administration of radioactive growth hormone preparations. *Endocrinology* 55:709-720, 1952
2. Elgee NJ, Williams RH: Specificity of insulin degradation reaction. *Diabetes* 4:87-91, 1955
3. Narahara HT, Everett NB, Simmons BS, Williams RH: Metabolism of Insulin-^{131}I and Glucagon-^{131}I in the kidney of the rat. *Am J Physiol* 192:227-231, 1958
4. Perri GC, Faulk M, Shapiro E, Money: Role of the kidney in the accumulation of egg-white muramidase in experimental animals. *Proc Soc Exp Biol Med* 115:189-192, 1963
5. Collip PJ, Patrick JR, Kaplan SA: Distribution of labelled human growth hormone in rats and guinea pigs. *Proc Soc Exp Biol Med* 121:173-177, 1966
6. Maack T: Changes in the activity of acid hydrolases during renal reabsorption of lysozyme. *J Cell Biol* 35:268-273, 1967
7. Maack T, Kinter WB: Transport of protein by flounder kidney tubules during long term incubation. *Am J Physiol* 216:1034-1043, 1969
8. De Luise M, Martin TJ, Melick RA: Tissue distribution of calcitonin in rat: comparison with parathyroid hormone. *J Endocrinol* 48:173-179, 1970
9. Hansen NE, Karle H, Andersen V: Lysozyme turnover in the rat. *J Clin Invest* 50:1473-1477, 1971
10. Maack T, Mackensie DDS, Kinter WB: Intracellular pathways of renal absorption of lysozyme. *Am J Physiol* 221:1609-1616, 1971
11. Maack T, Sigulem D: Renal handling of lysozyme, in *Lysozyme*, edited by Osserman EF, Proceedings of the International Conference on Lysozyme, Institute of Cancer Research, College of Physicians and Surgeons, Columbia University, Arden House, 1972, New York and London, Academic Press, Inc. 1974, p. 321-333
12. Maack T: Renal handling of low molecular weight proteins. *Am J Med* 58:57-64, 1975
13. Johnson V: The renal handling of growth hormone. Thesis, Graduate School of Medical Sciences, Cornell University Medical College, New York, 1976
14. Sigulem D: Contribuicao para o estudo do manejo de insulina pelo rim de rato. Thesis, Escola Paulista de Medicina, Sao Paulo, Brazil, 1976

15. JOHNSON V, MAACK T: Renal extraction, filtration, absorption and catabolism of growth hormone. *Am J Physiol* 233:F185–F196, 1977
16. KAU ST, MAACK T: Transport and catabolism of parathyroid hormone in isolated rat kidney. *Am J Physiol* 233:F444–F454, 1977
17. OTTOSEN PD, MAUNSBACH AB. Transport of peroxidase in flounder kidney tubules studied by electronmicroscope histochemistry. *Kidney Int* 3:315, 1973
18. DE KRETSER DM, MARTIN TJ, MELLICK RA. The autoradiographic localization of ^{125}I-labelled bovine parathyroid hormone. *J Endrocrinol* 46:507–510, 1970
19. DE KRETSER DM, CATT KJ, BURGER HG, SMITH GC. Radioautographic studies on the localization of ^{125}I-labelled human luteinizing and growth hormone in immature male rats. *J Endocrinol* 43:105, 1970
20. BECK LV, FEDYNSKY JN: Evidence from combined immunoassay and radioautography procedures that intact insulin-^{125}I molecules are concentrated by mouse kidney proximal tubule cells. *Endocrinology* 81:475–485, 1967
21. STRAUS W: Occurrence of phagosomes and phago-lysosomes on different segments of the nephron in relation to the reabsorption, transport, digestion, and extrusion of intravenously injected horseradish peroxidase. *J Cell Biol* 21:295– 308, 1964
22. STRAUS W: Colorimetric analysis with N, N-dimethyl-p-phenilene-diamine of the uptake of intravenously injected horseradish peroxidase by various tissues of the rat. *J Biophys Biochem Cytol* 4:541, 1958
23. ZULL JE, REPKE DW. The tissue distribution of tritiated parathyroid hormone in thyroparathyroidectomized rats. *J Biol Chem* 247:2195–2199, 1972
24. MILLER F, PALADE GE: Lytic activities in renal protein absorption droplets. An electron microscopical cytochemical study. *J Cell Biol* 23:519–552, 1964
25. MAUNSBACH AB: Absorption of ^{125}I-homologous albumin by rat kidney proximal tubular cells. *J Ultrastruct Res* 15:197–241, 1966
26. STRAUS W: Cytochemical observations on the relationship between lysosomes and phagosomes in kidney and liver by combined staining for acid phosphatase and intravenously injected horseradish peroxidase. *J Cell Biol* 20:497–507, 1964
27. BORDEAU JE, CHEN ERY, CARONE FA: Insulin uptake in the renal proximal tubule. *Am J Physiol* 225:1399–1404, 1973
28. CHRISTENSEN EI, MAUNSBACH AB: Intralysosomal digestion of lysozyme in renal proximal tubular cells. *Kidney Int* 6:396, 1974
29. STACY BD, WALLACE ALC, GEMMEL RT, WILSON BW. Absorption of ^{125}I-labelled sheep growth hormone in single proximal tubules of the rat kidney. *J Endocrinol* 68:21–30, 1976
30. NORQUIST RE, PALMIERI GMA: Intracellular localization of parathyroid hormone in the kidney. *Endocrinology* 95:229–237, 1974
31. MAACK T, MACLEOD M, SUMPIO B: Characteristics of a non-filtering isolated rat kidney preparation. *Fed Proc* 37:288, 1978
32. RENKIN EM, GILMORE JP: Glomerular filtration, in *Handbook of Physiology,* Renal Physiology, edited by ORLOFF J, BERLINER RW, American Physiological Society, Washington D.C., 1973, pp. 185–248
33. BRENNER BM, HOSTETTER TH, HUMES HD: Glomerular permselectivity: Barrier function based on discrimination of molecular size and charge. *Am J Physiol* 234:F455–F460, 1978
34. MARSHALL ME, DEUTCH HF. Clearance of some proteins by the dog kidney. *Am J Physiol* 163:461–467, 1950
35. LAMBERT PP, GREGOIRE F, MALMENDIER C, VANDERVEIBEN F, GRIERITTE G: Recherches sur le mechanism de l'albuminurie. *Bull Acad Roy Med Belg* 22:524–602, 1957
36. HARRISON JF, BARNES AD: The urinary excretion of lysozyme in dogs. *Clin Sci* 38:533–547, 1970
37. MAACK T: Reabsorptive transport of intact protein in the dog kidney. *Abst Proc IV Int Cong Nephrol* 1969, p. 296
38. MAACK T: Handling of proteins by the normal kidney. *Am J Med* 56:71–82, 1974
39. GREGOIRE F, LAMBERT PP: The biosynthesis of labelled Bence Jones protein and its glomerular permeability in the normal dog. *Clin Sci* 25:243–248, 1963
40. JOHNSON V, SIGULEM D, MAACK T: Characteristics of renal absorption of filtered protein (Lysozyme-LY). *Abst Proc 6th Cong Am Soc Nephrol* 1973, p. 54
41. DE MELLO G, MAACK T: Nephron function of the isolated perfused rat kidney. *Am J Physiol* 231:1699–1707, 1976
42. HRUSKA KA, KOPELMAN R, RUTHERFORD WE, KLAHR S, SLATOPOLSKI E: Metabolism of immunoreactive parathyroid hormone in the dog. *J Clin Invest* 56:39–48, 1975
43. WALLACE RD, STROBER W, THORBURN GD: The fate of radioiodinated sheep growth hormone in intact and nephrectomized sheep. *Pfluegers Arch* 331:25–27, 1972
44. WALDMANN TA, STROBER W, MOGIELNICKI RP: The renal handling of low molecular weight proteins: II. Disorders of serum protein catabolism in patients with tubular proteinuria, the nephrotic syndrome, or uremia. *J Clin Invest* 51:2162, 1972
45. HANSEN NE, KARLE H, ANDERSEN V, OLGAARD K: Lysozyme turnover in man. *J Clin Invest* 51:1146–1155, 1972
46. RENNKE HG, PATEL Y, VENKATACHALAM MA: Effect of molecular charge on glomerular permeability to proteins in the rat: clearance studies using neutral, anionic and cationic horseradish peroxidase. *Kidney Int* 13:278–288, 1978
47. COHN DV, MACGREGOR RR, SINHA D, HUANG DWY, EDELHOCH H, HAMILTON JW: The migration of proparathyroid hormone, parathyroid hormone and their peptide fragments during gel filtration. *Arch Biochem Biophys* 164:669–673, 1974
48. VON MOLLENDORF W. Die dispersität der farbstoffe, ihre Beziehungen sur Speciherung und Ausscheidung in der Niere. *Anat Hefte* 53:81–323, 1915
49. GERARD P, CORDIER R: Esquisse d'une histophysiologie comparee du rein des vertebres. *Biol Rev Cambridge Phil Soc* 9:110–131, 1934
50. OLIVER J: New directions in renal morphology: A method, its results and the future. *Harvey Lect* 40:102–115, 1944
51. SMETANA H, JOHNSON FR: The origin of colloid and lipid droplets in the epithelial cells of the renal tubules. *Am J Pathol* 18:1029–1049, 1942
52. YUILE CL, CLARK WF: Myoglobinuria: A study of the renal clearance of myoglobin in the dog. *J Exp Med* 74:187–196, 1941
53. ARNOULD Y, OOMS HA, FRANCKSON JRM. Analysis of urinary excretion of insulin in the normal dog. *Arch Int Pharmakodyn* 167:480–482, 1967
54. CORTNEY MA, SAWIN LL, WEISS DD: Renal tubular protein absorption in the rat. *J Clin Invest* 49:1–4, 1970
55. LANDWEHR DM, CARVALHO JS, OKEN DE: Micropuncture

studies of the filtration and absorption of albumin by nephrotic rats. *Kidney Int* 11:9–17, 1977
56. Baldamus CA, Galaske RG, Eisenbach GM, Krause HP, Stolte H: Glomerular protein filtration in normal and nephrotic rats: a micropuncture study, in *Contributions to Nephrology,* Basel/New York, S. Karger, 1975, vol. 1, pp. 37–49
57. Solling K, Mogensen CE, Vittinghus E, Brock A: The renal handling of amylase in normal man. *Abst Proc VII Int Cong Nephrol* 1978, p. X-4
58. Straus W: Colorimetric investigation of the uptake of intravenously injected protein (horseradish peroxidase) by rat kidney and effects of competition with egg-white. *J Cell Biol* 12:231, 1962
59. Harrison JF, Blainey JD: Low molecular weight proteinuria in chronic renal disease. *Clin Sci* 33:381, 1967
60. Cohn ZA: The regulation of pinocytosis in mouse macrophages. *J Exp Med* 124:557–571, 1966
61. Kau ST, Maack T: Effect of cytochalasin B and decreased sodium reabsorption on tubular uptake of protein (abst). *Fed Proc* 36:577, 1977
62. Peterson DR, Oparil S, Flouret G, Carone FA: Handling of angiotensin II and oxytocin by renal tubular segments perfused in vitro. *Am J Physiol* 232:F319–F324, 1977
63. Piscator M: Proteinuria in chronic cadmium poisoning. *Arch Environ Health* 5:325–332, 1962
64. Prockop DJ, Davidson WD: A study of urinary and serum lysozyme in patients with renal disease. *N Engl J Med* 270:269–274, 1964
65. Balazs T, Roepke RR: Lysozymuria induced in rats by nephrotoxic agents. *Proc Soc Exp Biol Med* 123:380–385, 1966
66. Harrison JF, Lunt GS, Scott LP, Blainey JD: Urinary lysozyme, ribonuclease and low molecular weight proteins in renal disease. *Lancet* 1:371, 1968
67. Hayslett JP, Perillie PE, Finch SC: Urinary muramidase and renal disease. *N Engl J Med* 279:506–512, 1968
68. Peterson PA, Evrin PE, Berggard I: Differentiation of glomerular, tubular and normal proteinuria: Determinations of urinary excretion of B_2 microglobulin, albumin and total protein. *J Clin Invest* 48:1169–1198, 1969
69. Pruzanski W, Platts ME: Serum and urinary proteins, lysozyme (muramidase), and renal dysfunction in mono- and myelomonocytic leukemia. *J Clin Invest* 49:1694–1708, 1970
70. Mogielnicki RP, Waldmann TA, Strober W: Renal handling of low molecular weight proteins I. L-chain metabolism in experimental renal disease. *J Clin Invest* 50:901–909, 1971
71. Christensen EI: Rapid protein uptake and digestion in proximal tubule lysosomes. *Kidney Int* 10:301–310, 1976
72. Wochner RD, Strober W, Waldmann TA: The role of the kidney in the catabolism of Bence Jones proteins and immunoglobulin fragments. *J Exp Med* 126:207–220, 1967
73. Koss MN, Pirani CL, Osserman EF: Experimental Bence Jones cast nephropathy. Lab Invest 34:579–591, 1976
74. Hruska KA, Martin K, Mendes A, Anderson C, Klahr S, Slatopolski E: Degradation of parathyroid hormone and fragment production by the isolated perfused dog kidney. *J. Clin Invest* 60:501–510, 1977
75. Martin K, Hruska KA, Lewis J, Anderson C, Slatopolski E: The renal handling of parathyroid hormone in the dog: The role of peritubular uptake and glomerular filtration. *J Clin Invest* 60:808–814, 1977
76. Katz AI, Rubenstein AH: Metabolism of proinsulin, insulin and C-peptide in the rat. *J Clin Invest* 52:1113–1121, 1973
77. Muggia FM, Heinemann HO, Farhangi M, Osserman EF: Lysozymuria and renal tubular dysfunction in monocytic and myelomonocytic leukemia. *Am J Med* 47:351–365, 1969
78. Dohi SR, Terzian JF, Widman A, Brentani R, Fausto N, Liberman B, Rabinovitch M: Inactivation of serum ribonuclease by the kidney. *Am J Physiol* 196:924–926, 1959
79. Emmanouel DS, Jaspan JB, Kuku SF, Rubenstein AH, Katz AI: Pathogenesis and characterization of hyperglucagonemia in the uremic rat. *J Clin Invest* 58:1266–1279, 1976
80. Rabinovitch M, Dohi SR: Increase in serum ribonuclease activity after bilateral nephrectomy. *Am J Physiol* 187:525–528, 1956
81. Houck JC, Berman LB: Serum ribonuclease activity. *J Appl Physiol* 12:473, 1958
82. Keeler R: The effect of bilateral nephrectomy on the production and distribution of muramidase (lysozyme) in the rat. *Can J Physiol Pharmacol* 48:131–138, 1970
83. Wallace ALC, Stacy BD: Disappearance rate of ^{125}I-labelled rat growth hormone in nephrectomized and sham operated rats. *Horm Metab Res* 7:135–138, 1975
84. Wright AD, Lowy C, Frazer TR, Spitz IM, Rubenstein AH, Bersohn I: Serum growth hormone and glucose tolerance in renal failure. *Lancet* 2:798–801, 1968
85. Martin TJ, Melick RA, deLuise M. The effect of nephrectomy on the metabolism of labelled parathyroid hormone. *Clin Sci* 37:137–142, 1969
86. Melick RA, Martin TJ: Parathyroid hormone metabolism in man: Effect of nephrectomy. *Clin Sci* 37:557–674, 1969
87. Bastl C, Finkelstein FO, Sherwin R, Hendler R, Felig P, Hayslett JP: Renal extraction of glucagon in rats with normal and reduced renal function. *Am J Physiol* 233:F67–F71, 1977
88. Sherwin R, Bastl C, Finkelstein FO, Black H, Hendler R, Felig P: Influence of uremia and hemodialysis on the turnover and metabolic effects of glucagon. *J Clin Invest* 57:722–731, 1976
89. Raunskov B, Karatson A: Renal handling of human B_2 microglobulin in the rat: The importance of sham operation. *Acta Physiol Scand* 94:467–471, 1975
90. Ricketts HT, Wildberger HL, Regut L: The role of the kidney in the disposal of insulin in rats. *Diabetes* 2:155–161, 1963
91. Rabkin R, Colwell JA: The renal uptake and excretion of insulin in the dog. *J Lab Clin Med* 73:893–900, 1969
92. Canterbury JM, Reiss E: Multiple immunoreactive molecular forms of parathyroid hormone in human serum. *Proc Soc Exp Biol Med* 140:1393–1398, 1972
93. Habener JF, Powell D, Murray TM, Mayer GP, Potts JT Jr: Parathyroid hormone: Secretion and metabolism in vivo. *Proc Natl Acad Sci USA* 68:2986, 1971
94. Chamberlain MJ, Stimmler L: The renal handling of insulin. *J Clin Invest* 46:911–919, 1967
95. Rubenstein AH, Spitz I: Role of the kidney in insulin metabolism and excretion. *Diabetes* 17:161–169, 1968