Letters to the Editor

Proteinuria in Diabetes

Dear Sir,

We would like to make a few comments of a technical nature concerning the statements in the letter by Dr. Simpson in the August 1982 issue [1]. When assessing proteinuria as a marker of glomerular function, it is essential to quantitate specifically and accurately one or more proteins, the renal handling characteristics of which are known and understood. Albumin is the obvious choice as it is the major filtered plasma protein in normal urine, and in mildly elevated 'Albustix'-negative proteinuria (total protein excretion <0.5 g/24 h) comprises 7%–15% of the total. Moreover, in 'Albustix'-positive clinical proteinuria (total protein excretion >0.5 g/24 h) albumin is the major protein component of any origin, representing 40%–50% of the total [23]. The filtration of albumin is greatly restricted by the glomerulus. In the rat, the plasma concentration is about 40 g/l, that found in the Bowman space is only 15 mg/l. Of this approximately 90% is reabsorbed by the proximal tubules [4]. However, the reabsorption process is easily saturated by relatively small increases in filtration [5]. Even under conditions of non-saturation the excretion of albumin in the urine should change in proportion to the amount filtered if, as seems likely, the reabsorption of proteins is proportional to the filtered load [6]. Therefore, as a general rule, urinary excretion reflects glomerular filtration.

Using sodium dodecyl sulphate polyacrylamide gel electrophoresis to establish 'patterns' of proteins (unidentified save by molecular weight) in normal urine, and expecting to make definitive statements concerning the properties of the glomerular filter, is unrealistic. Though this technique has been used in 'clinical' proteinuria to provide information supplementary to albumin measurements, one must be extremely cautious when applying this technique to normal or mildly elevated proteinuria. Under these circumstances, by far the greatest proportion of the protein reaches the urine by *post-glomerular secretion* and has absolutely no relevance to glomerular filtration or the nature of the glomerular barrier. These *secreted* proteins include the dimeric secretory form of IgA (molecular weight 385,000) believed to be secreted by the tubules; urokinase, a fibrinolytic enzyme (molecular weight 55,000) found only in urine and not in plasma; B-galactosidase (molecular weight 230,000), found in normal urine in similar quantities to albumin; and, most important of all, the Tamm-Horsfall mucoprotein, which alone comprises 30%–50% of the total protein in normal urine [7]. This is a very large molecule (molecular weight 23,000,000) excreted by the distal convoluted tubules and the loops of Henle [8]. This protein has been shown to be disaggregated into individual subunits with a molecular weight of 80,000–100,000 by a number of agents including sodium dodecyl sulphate [9]. Normal urine also contains a large proportion of secreted heterogenous mucoproteins which are yet to be adequately characterised.

We must stress, therefore, that without further analysis of these high molecular weight proteins (170,000–215,000), noted by Dr. Simpson in the urine of different groups of subjects with otherwise normal kidney function, he cannot make inferences about changes at the glomerular filtration level.

Diabetic kidney disease is primarily a glomerulopathy, and proteinuria has recognised clinical importance as an early indicator of this pathological process. Thus in a study of diabetic proteinuria the main line of approach should involve methods, the results of which can be interpreted unambiguously to provide insights into the nature of the glomerular lesion(s), leaving other techniques as a possible supplementary approach.

Yours sincerely,
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References

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Insulin Does Not Aggregate Its Own Receptor

Dear Sir,

The concept that insulin can aggregate its own receptor is used by Jeffrey [1] to formulate a hypothesis of the mechanism of insulin action. However, experiments [2] using insulin labelled with ferritin as an ultrastructural marker for the insulin receptor, do not support the idea of hormone-induced receptor aggregation. The validity of monomeric ferritin-insulin (Fm-I) as a receptor marker is based on studies [3] demonstrating that it binds specifically to the insulin receptor, is equivalent in immunological reactivity to insulin, and stimulates glucose oxidation to the same extent as native insulin.

Fm-I occupied receptor sites on adipocyte plasma membranes occur in the natural state as groups of two to six molecules [4]. Pre-fixation of the membranes before the addition of Fm-I does not change the pattern of receptor distribution [4], and therefore argues against a ligand-induced receptor aggregation.

In contrast to adipocyte membranes, Fm-I occupied receptors on liver membranes exist predominantly as single sites [2]. This distribution pattern is not altered over a fourfold range of Fm-I concentration [5], thus further upholding the counter-proposal that insulin does not aggregate its own receptor.

The fluorescence microscopy observations [6] that are often quoted in support of the notion of receptor aggregation require re-appraisal. Unlike Fm-I which has nearly 100% of insulin's activity, rhoda-