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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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-

mine conjugated insulin (rhodamine-lactalbumin-insulin) used for these studies retains <2% of the activity of native insulin [7]. It is therefore difficult to see how one can arrive at physiologically significant conclusions from these experiments. Furthermore, the fluorescence is visualized using image intensification microscopy, and the clustering observed could be an order of magnitude greater than the micro-aggregation seen by electron microscopy. Hence the data obtained with rhodamine-insulin might not be comparable to that of Fm-I, and molecular models of receptor aggregation [1] may not be applicable to these fluorescence studies.

Jeffrey [1] also cites the work of Kahn et al. [8] to support the tenet of insulin-induced receptor aggregation. Monovalent Fab' fragments of anti-receptor antibody were shown to bind to the insulin receptor but were unable to stimulate glucose oxidation. Addition of anti-Fab' fragments restored the ability of the Fab' fragments to stimulate glucose oxidation. Kahn et al. concluded [8] that anti-receptor antibody acts by aggregating the insulin receptor. However, their data may have alternative interpretations. For instance, one can postulate that there are separate binding and biological response sites for the insulin receptor, with corresponding complementary sites for the insulin molecule and the anti-receptor antibody. When the anti-receptor antibody is split into its Fab' fragments, a minor conformational change might occur, resulting in the retention of binding activity, but loss of the ability to stimulate biochemical activity. The addition of anti-Fab' antibody can perhaps expose the sites for biological activity on the Fab' fragments by restoring the original conformation. There is then no need to invoke receptor aggregation to explain the biochemical activity of the anti-receptor antibody or insulin.

Any hypothesis concerning the role of groups of insulin receptors must account for all the pertinent morphological and biochemical observations including the following. First the natural state of distribution of insulin receptors appears to be tissue specific [2, 4, 9]. Secondly dithiothreitol, a reducing agent, disperses the groups of Fm-I occupied receptors on adipocyte membranes [10], yet increases ¹²⁵I-insulin binding to these membranes about threefold [11]. Thirdly anti-insulin antibody [5] induces aggregation of the largely single Fm-I occupied receptors on liver membranes, and causes up to a 15-fold increase in ¹²⁵I-insulin binding to liver membranes [12].

In conclusion, the validity of the interpretations from experimental data [6, 8] used to support the concept of insulin-induced receptor aggregation, can be questioned. There are experimental findings to endorse the opposite view that insulin does not aggregate its own receptor [2, 4, 5]. Receptor aggregation, nevertheless, does occur in the natural state of certain tissues [2, 9] and may not be necessary as a primary mechanism for insulin action. However, there might be a secondary role for receptor aggregation in modulating the biochemical responses to insulin [5, 11, 12]. Studies are needed to determine whether the pattern of aggregation of the insulin receptor seen in the natural state is an intrinsic property of the receptor structure or the membrane micro-environment for each individual tissue.

Yours sincerely,
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Antiplatelet /Anticoagulant Drug Therapy in Severely Uncontrolled Diabetes Mellitus

Dear Sir,

We wish to comment on the Letter to the Editor by Janka and Mehnert [1] on the role of antiplatelet agents in diabetic ketoacidosis. There have recently been several reports of haemostatic changes in diabetic coma [2, 3] and, while a coagulopathy may exist, its association with fatal thromboembolic events has not been established. In the hope of decreasing mortality from fatal thromboembolism and, in turn, lowering overall mortality from uncontrolled diabetes (up to 50% in the elderly [4]) some investigators have proposed prophylactic anticoagulation in these patients [5–8]. This has gained increased support in the literature and while antiplatelet drugs are not recommended, it has been suggested that heparin may be warranted in high risk uncontrolled diabetic patients. In a recent review of 275 cases of uncontrolled diabetes (unpublished observations), we noted one cerebrovascular accident in a hyperosmolar patient as well as one mesenteric arterial occlusion and one cerebrovascular accident in ketoacidotic patients. In reviewing several large series and case reports including 251 cases of hyperosmolar coma and 1,764 cases of ketoacidosis, we found that major fatal or near fatal thromboembolic (pulmonary embolus, cerebrovascular occlusion, mesenteric arterial occlusion or limb loss due to arterial occlusion) represented less than 1% of events in ketoacidosis and 6% in hyperosmolar coma (references available on request). Many of these events occurred with severe hypotension and might just as easily be explained by increased serum viscosity with low flow in an already compromised circulation rather