

Is the Albumin Retrieval Hypothesis a Paradigm Shift for Nephrology?

Anthony G.W. Norden, and Robert J. Unwin

Department of Clinical Biochemistry, Addenbrooke's Hospital Cambridge and UCL Centre for Nephrology, University College London Medical School, Royal Free Campus and Hospital, London, United Kingdom

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The albumin retrieval hypothesis is alive and well, if still controversial. Although emphasis on different aspects of this hypothesis has changed over the years, there are now several papers in the nephrology and clinical chemistry literature that seem to endorse it.^{1,2} In its original form, the hypothesis depended on three related concepts: albumin is more freely filtered by the renal glomerulus than we had thought previously; large quantities of filtered albumin are reabsorbed by the proximal tubule; and large amounts of reabsorbed albumin are returned intact to the circulation, as well as being degraded by lysosomes in the proximal tubule to appear as fragments in the final urine.

Of course, quantifying these processes in different species, defining their anatomic correlates, and identifying the molecular transporters involved are all essential in testing fully this hypothesis. Moreover, if it can withstand these tests, there will be a variety of human renal diseases for which conventional pathophysiology is wrong, or at least incomplete, switching the focus of disease and pathophysiology from the glomerulus to the tubule.

In this issue of *JASN*, Weyer *et al.*,³ members of the Aarhus group, which is well known for its seminal work on the handling of low-molecular-weight (LMW) proteins by the proximal tubule, have tackled in part the second and third of the underlying concepts listed above: the generation of putative fragments of plasma albumin in urine. They have used the mouse and their own elegant transgenic models of conditional megalin and cubilin, or megalin only null mice.⁴ Curiously, Weyer *et al.* do not address the question of whether there are indeed significant quantities of such degraded albumin fragments in mouse urine or their nature; in fact, the molecular

identities and quantities of these fragments are still unknown in any species.

The reliability of the measurements reported in at least one of the references cited in support of the existence of large quantities of albumin fragments in human urine has been questioned.^{5,6} However, in their current paper, the Aarhus group propose that the origins of these albumin fragments, if they are really generated by the kidney, must depend on the integrity of the megalin–cubilin pathway.⁴ This pathway appears to be the dominant albumin transporter and uptake mechanism in the proximal tubule, and if this is true, the null mice should not have any albumin fragments in their urine. As it happens, the urine of these mice did contain albumin fragments, at least of its radiolabeled form, and the interpretation of this is that they are prerenal in origin—generated in the circulating plasma and filtered—because a postrenal source seems unlikely.

The technique they use to detect putative albumin fragments in urine is to inject rats with chloramine-T radio-iodinated albumin and to divide the radioactivity of urine into high-molecular-weight (HMW) and LMW fractions by size exclusion chromatography on a Sephadex G-100 column. HMW fractions are taken to comprise intact albumin and LMW fractions to contain albumin fragments; however, without providing column calibration data, we cannot be sure of the molecular size of the LMW fractions. Indeed, it is not certain that a contribution from free iodide has been excluded. We also cannot reliably assess the quantities of albumin fragments from the relative amounts of radioactivity in LMW and HMW fractions, although Weyer *et al.* estimate that approximately 1% of albumin in plasma is found in LMW form.

Recovery data for the chromatography experiments are not presented, and because only small quantities of protein were applied to relatively large columns, adsorption losses are possible. Furthermore, proteins radiolabeled with chloramine-T are known to suffer oxidative damage⁷ and to be endocytosed abnormally in some systems.⁸ Chromatography of the radio-iodinated albumin used for injection demonstrates some heterogeneity, possibly caused by aggregation (Figure 2D of ref. 3). However, even with these potential sources of error, the results of this study still make it unlikely that large quantities of albumin fragments generated by the kidney by a megalin–cubilin-dependent pathway would have been missed.

Where do these new findings sit in relation to previously published work and how do they relate to the albumin retrieval hypothesis? There has been an analogous approach to the same question in humans. Dent's disease, a renal Fanconi syndrome, has been used as a functional human megalin–cubilin knockout.⁶ Similar to Weyer *et al.*, albeit using chemical methods and not radiolabeling, a change in any putative urine albumin fragment was looked for but was not

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Correspondence: Dr. Robert John Unwin, University College London, Centre for Nephrology, Royal Free and University College Medical School, Rowland Hill Street, London NW3 2PF, United Kingdom. Email: robert.unwin@ucl.ac.uk

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found. Of course, negative experimental results of this sort are inherently unsatisfactory, and there is always a nagging doubt that something has been missed theoretically or methodologically. Rather than an accumulation of negative experimental data, what other approaches might be useful? It seems of key scientific and clinical importance in nephrology to settle the many questions raised by the albumin retrieval hypothesis, but we need a secure theoretical framework with which to move forward.

We need rigorous measurements and to be aware of inter- and intraspecies differences. An essential element of the albumin retrieval hypothesis is the proposal that glomerular permeability to albumin is high.¹ Proponents of the hypothesis suggest high albumin permeability, with a glomerular sieving coefficient (GSC) for albumin of approximately 0.035, which predicts that the normal human kidney handles approximately 240 g of filtered albumin/d.¹ In contrast, an indirect estimate of the GSC for albumin in humans is nearer 0.0001, which is more than two orders of magnitude less⁹; both estimates cannot be correct. Large quantities of albumin fragments and albumin transcytosis depend on a high GSC for albumin. It is not clear whether anatomical measurements of pore size can settle this.^{10,11}

Potentially, the most productive approach will be to make measurements of albumin permeability in animal models and to correlate these with altered glomerular barrier function in transgenic models.¹² What does it mean that despite the findings of several two-photon microscopy studies, there are still major disagreements over the measurements of albumin GSC?^{2,13–15} A problem with the two-photon approach is that it is difficult to calibrate and to avoid interference from adjacent structures. The results are at the limit of what can be achieved with current technology, which makes them particularly challenging. A combination of genetic knockouts and physical measurements would be useful, but unfortunately there is still only a limited prospect of generating suitable genetic models in the rat, the species in which two-photon measurements have been made.

Are micropuncture studies worth revisiting and could they be combined with the two-photon approach? Available micropuncture data in the rat comes from a single report by Tojo and Endou.¹⁶ The pioneering anatomic studies of Maunsbach¹⁷ are often used to challenge the albumin retrieval hypothesis, although this evidence is perhaps still insufficient. Comparison of two-photon and micropuncture results, possibly from the same glomerulus, might be the best we can achieve technically. Triangulation, by adding a third independent variable, may have to wait until glomerular-null rodents become available for study. We suspect that matters will remain unresolved for some time to come, at least until there is a more standardized approach to the use and application of the two-photon technique in investigating this issue.

More rigorous physical measurements in rodents and humans should be used to define the exact molecular species and the quantity of albumin fragments in urine. Proteomic and peptidomic techniques are making such rapid advances that this should be straightforward. The albumin retrieval hypothesis

rests critically on the belief that albumin filtration, and thereby the quantity of albumin fragments, is large.¹

Transcytosis of large quantities of albumin is a more difficult problem. Weyer *et al.* did not design their experiments to investigate this specifically, and it is always possible to invoke technical reasons for a failure to detect large quantities of albumin: the vesicles are too small to see or their transit is too rapid, although the physical boundaries remain. If there really is transcytosis of large quantities of albumin, at least in rodents, we should be able to freeze the process and find significant amounts of albumin in various states of processing in the kidney.

In summary, while the work of Weyer *et al.* has addressed one important aspect of the albumin retrieval hypothesis, we suggest three additional ways in which the unanswered questions might be tackled: a collaborative effort to measure the GSC of albumin in rats and to correlate measurements with micropuncture data; further proteomic and peptidomic approaches in rodent urine and in humans; and in mice to correlate the results with transgenic models. As a corollary, measurements of albumin, and possibly modified forms of albumin being transcytosed within the kidney, should also be made.

Jared and Miner¹³ have asked for the definitive experiment and data to settle the matter. However, we doubt there can be a single experiment or that the necessary data can be obtained easily with the available techniques. What we need is the GSC or range of GSCs for albumin in normal humans. Current indirect measurements, which do not define the site of albumin selectivity, are inadequate, even if they set a boundary or range for the GSC for albumin in humans.⁹ Molitoris¹¹ has likened the albumin retrieval hypothesis to the philosophical paradigm shift in modern physics that occurred when quantum mechanics transformed classic Newtonian mechanics. However, we need more observations and experiments before that comparison can be applied to the renal handling of albumin.

DISCLOSURES

None.

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See related article, "Generation of Urinary Albumin Fragments Does Not Require Proximal Tubular Uptake," on pages 591–596.

The Eternal (Nocturnal) Quest for Better Dialysis Outcomes

Martin K. Kuhlmann

Department of Internal Medicine, Division of Nephrology, Vivantes Klinikum im Friedrichshain, Berlin, Germany

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Twenty years ago, Bernard Charra and colleagues from Tassin, France, published a seminal paper on the survival rate of patients undergoing thrice weekly 8-hour in-center

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Correspondence: Dr. Martin K. Kuhlmann, Department of Internal Medicine, Division of Nephrology, Vivantes Klinikum im Friedrichshain, Landsberger Allee 49, D-10249 Berlin, Germany. Email: martin.kuhlmann@vivantes.de

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hemodialysis (HD) treatments.¹ This publication, which still represents the gold standard for outcome in thrice weekly dialysis regimens, induced a global search for improved dialysis regimens leading to better outcomes. Twenty years, millions of dollars, and a multitude of clinical studies later, the quest for improved dialysis outcomes is still ongoing.

The first decade since 1992 was mainly Kt/V-oriented, with clinical studies trying to define an optimum dialysis dose with respect to the diffusive elimination of small water-soluble uremic compounds. Eventually, the HEMO study, a randomized controlled trial comparing sessional target Kt/Vs of 1.20 and 1.45, put an end to this discussion by demonstrating in thrice weekly conventional HD that increasing sessional target Kt/V beyond 1.2 did not improve survival further.² Recognition of these obvious limitations of a urea-centered dialysis world led to renewed interest in the removal of other potentially relevant azotemic toxins such as β -2-microglobulin, phosphate, and middle molecules.

At the beginning of the second decade after Tassin, the research focus shifted toward convection, dialysis length, and treatment frequency. Several randomized controlled studies comparing hemodiafiltration (HDF) with conventional low or high-flux HD were initiated, which have either recently been completed or are still ongoing. Data from two of those studies, the Dutch Convective Transport Study and a Turkish study, have been presented in oral or abstract form in 2011, indicating that increasing convection by thrice weekly 3- to 5-hour on-line HDF had no significant effect on the outcome of dialysis patients.

At the same time, there was an accumulation of encouraging data from small controlled or larger observational studies on the positive effects of more intense dialysis regimens on patient satisfaction and outcome. To answer the crucial question, whether the outcome of dialysis patients is significantly improved by maximally increasing dialysis dose and frequency, the two-armed Frequent Hemodialysis Network (FHN) Study was initiated. The first arm of the study examined the effect of short daily in-center HD compared with conventional thrice weekly HD over a 12-month period and had a positive result with the two primary endpoints, mortality or increase in left ventricular mass, and mortality or decrease in physical health composite score, being significantly lower in the more frequent HD group.³ The second arm was designed to examine the effect of daily nocturnal, 6- to 8-hour home HD compared with conventional thrice weekly home HD. Although in the nocturnal FHN arm, the delivered dialysis dose was profoundly higher than in the short FHN arm, there was no effect on the same predefined primary outcome parameters.⁴ The nocturnal FHN arm suffered from a slow and difficult recruiting process, which allowed only 87 patients to be randomized and thus may be considered severely underpowered.⁵ Because the FHN nocturnal study was not able to give the desired final answer on dialysis dosing, the book on nocturnal HD is not closed.

Twenty years after the Charra publication, it appears that there is a revival of thrice weekly in-center nocturnal HD, not only in Europe, but also in the United States, where, for example,