LETTERS TO THE EDITOR

Are fractional clearances overestimated?

To the Editor: A recent paper by Burne, Osicka, and Comper [1] seems to have serious flaws that deserve attention. The authors present fractional clearances, $\theta$, of high molecular weight proteins in conscious rats that are considerably higher than expected. Rather than reflecting over their own experimental technique the authors conclude that all previous studies are false! Is it not more likely that the $\theta$ values are overestimated due to protein degradation in vivo? Indeed, Burne, Osicka, and Comper suggest that more than 99.997% of the proteins in plasma are intact on the basis of two (of their own) papers. However, those values were obtained after days of dialysis of the albumin tracer in vitro at 4°C. Is that not a situation dramatically different from that in vivo, where, for example, proteases are present?

In fact, it is absolutely crucial to avoid small amounts of degraded material in plasma, since 0.2 to 0.4% could account for most of the degraded large proteins found in urine [2]. There are several published papers on isolated perfused kidneys showing considerable size- and charge-selectivity for proteins both in the small- and the large-pore pathways [2–4]. In those studies the tubular uptake and protease activities were inhibited by low temperature and the urine analyzed for intact proteins. The results, that is, $\theta$ for albumin around 0.1%, seem to disprove the authors’ hypothesis of albumin having “true” glomerular $\theta$ of 6% (!) and massive tubular uptake of intact protein [5]. Incidentally, the latter mechanism is in conflict with several other (not cited) observations [6]. From a scientific point of view it is interesting that neither the authors nor the reviewers have found it necessary to even mention these studies.

Finally, Burne, Osicka, and Comper conclude that there is “a weak dependence of $\theta$ on size-selective filtration, except for albumin, which undergoes a specific type of postglomerular processing.” It would be of interest for the readers to know how the authors can conclude this from the present experimental data with, for example, a fractional clearance for albumin of 0.2%?

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REPLY FROM THE AUTHORS

The issues that Dr. Haraldsson raises concerning plasma proteases and contamination have already been thoroughly addressed in a previous publication [1]. Ultrapure tritium-labeled albumin (all the labeled high molecular weight proteins used in our study [2] were prepared in the same way) was injected intravenously into a rat. Urine was collected over a period of 2 hours and the plasma collected for analysis at the end of the 2-hour period. The 2-hour plasma radioactivity was demonstrated to be 99.997% intact albumin as determined by size exclusion chromatography [1]. No low molecular weight contaminants, possibly formed by the action of proteases in plasma, could be detected. If they were present they would have been less than 0.003% of the material analysed (limit of our sensitivity). This is 100 times lower than the suggested contamination value by Dr. Haraldsson of ~0.3% (this level of contamination assumes that peptides are excreted freely, but we have recently demonstrated that fractional clearance of albumin peptides is ~0.003 [Jones and Comper, unpublished observation] which means that the potential for contamination is essentially negligible). On the other hand, the urine contained >95% fragmented albumin that could not have come from low molecular labeled species in the plasma. These studies demonstrated that filtered albumin in vivo is degraded during renal passage and that this is not due to any significant action of proteases in plasma on this molecule.

Parallel studies using the isolated perfused rat kidney perfused (IPK) at 37°C where there are no proteases present in the perfusate or in the urine also gave the same trend in clearances to those we reported [2], although the fractional clearances in the IPK are generally higher ($N = 4$ for all proteins, fractional clearances (fc) are presented as means ± sd: albumin fc = 0.0080 ± 0.0002; lactate dehydrogenase fc = 0.0165 ± 0.0042; glucose oxidase fc = 0.0110 ± 0.009 from [4]).