Methodological Problems in the Simultaneous Determination of *p*-Aminohippurate and Inulin in Water and Plasma: Is It Safe to Stock Samples for Future Determinations and Use Standard Curves for One Substance when Both Are Present in the Patient's Plasma?

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Dear Sir,

The 'golden standard' methods for the measurement of renal plasma flow and of glomerular filtration rate are, respectively, the clearances of *p*-aminohippurate (PAH) and of inulin. The traditional method requires catheterization of the bladder which, in addition to causing discomfort to the patient and to being potentially harmful, is not suitable when multiple assessments of renal plasma flow and glomerular filtration rate are necessary in a short period of time. It has been shown that from the serum levels of the substances and their infusion rate it is possible to calculate their clearances [1] and that this method shows a higher degree of correlation and a lesser degree of variance between multiple sampling periods, when compared with the traditional one [2]. When, for one of our research protocols, we used this method, we encountered two problems. (1) Is it possible to store the plasma samples for some time before analysis? It could be that PAH and/or inulin adsorb to plasma proteins or to the walls of the vials after some time. Since both methods require protein precipitation, these events could be of importance. (2) When PAH and inulin are mixed together in the syringe and are both present in plasma samples, is there an interference between these two compounds in their respective assays? Usually people rely for a long time on the same standard curve, done for a single compound, unless there is a change in reagents or in instrumentation, since both measurements have a good degree of reproducibility.

To answer these questions we constructed 4 standard curves for both PAH and inulin simultaneously present in water and in plasma, after 3 h from the preparation of the samples and after 96 h of storage at 4°C. Moreover,

Table 1. Results of the paired t tests between the different determinations

during the determinations of PAH and inulin clearances in a patient, the same samples of PAH and inulin both in water (infusion) and in plasma were read on 2 different standard curves: one only with the substance to be tested, and the other with the other substance too. PAH and inulin

clearances were then accordingly calculated. The assay methods we used, with slight modifications, are widely employed [3, 4].

As may be seen from the table 1 there are no significant differences in the determinations done 96 or 3 h after the preparation of samples. Moreover, the percentages of variation between assays are trivial (0.1–0.3%). When we compare instead the colorimetric determinations done with standard curves containing both PAH and inulin, with the colorimetric determinations done with standard

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curves containing only PAH or inulin, more divergent 2 results are obtained. In fact the range of variation is 1–4% and the consistency of divergency is significant for PAH and of borderline significance for inulin.

When finally we calculate the clearances of the two substances from the plasma levels calculated according to the 2 different curves, we discover that the divergency is even greater in percentage (3–5%) and is always statistically significant.

In conclusion, our results show that it is safe to stock at 4°C the samples for both inulin and PAH determination in water and plasma, but, if we are going to measure clearances of both substances together, it is more reliable to calculate the standard curves of both PAH and inulin with the presence of the other substance too, since otherwise there are risks of underestimating the clearances of the two compounds and the filtration fraction.

References

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