over three weeks with 10 urine samples having various albumin concentrations (*3*). Moreover, in a recent Letter (*4*) in reply to the article of Elving et al. (*2*), we reported the time courses of albumin values measured in three urine pools with two different RIA kits (*3, 5*). A significant, but very slight, decrease of albumin concentration (about 0.6 mg/L after six months) was found only in a low-concentration pool; the values of two intermediate-concentration pools did not decrease. We concluded that, although statistically significant, the decrease was of no clinical relevance (*5*).

Recently, Osberg et al. (*6*) reported significantly lower RIA measurements of albuminuria in samples stored at −20 °C than in fresh urine samples. Surprisingly, they stated: "Our present report is, to our knowledge, the first evaluation of the effects of freezing on albumin concentrations in urine as determined with a commonly available commercial radioimmunoassay kit" (*6*). Hence, we respond by referring to our previous studies and also report here further findings confirming that, in our experience (which, concerning RIA of albumin, covers six years at least), there is no significant decrease in the measurement of albumin concentration by RIA in urine samples stored for many weeks at −20 °C.

In Figure 1, we report the time-course of albumin measurements in five urine pools containing various albumin concentrations and stored in several aliquots at −20 °C until assay. We used these aliquots for the internal quality control of our RIA kit (Albumin RIA 100; Pharmacia AB, Uppsala, Sweden) (*5*) for measuring UAE through 1989 and 1990. Stability of all five pools is evident, as is the good reproducibility of our RIA (CVs 4–9%).

Moreover, starting in March 1988, we organized a National External Quality Control for the assay of urinary albumin in Italy. We sent frozen urine samples (n = 450) to the 15 laboratories participating in the survey. We did not observe any significant decrement of urinary concentration measured by the two RIA methods (H-Albumin, Sclavo, Cinisello Balsamo, Milano, Italy; and Albumin RIA 100) used by respectively seven and eight laboratories during the period of the study (*7*).

How can one explain the discrepancies between our results and the results of Elving et al. (*2*) and Osberg et al. (*6*)? At present, we provide some suggestions only. As we already pointed out (*4*), differences in method-

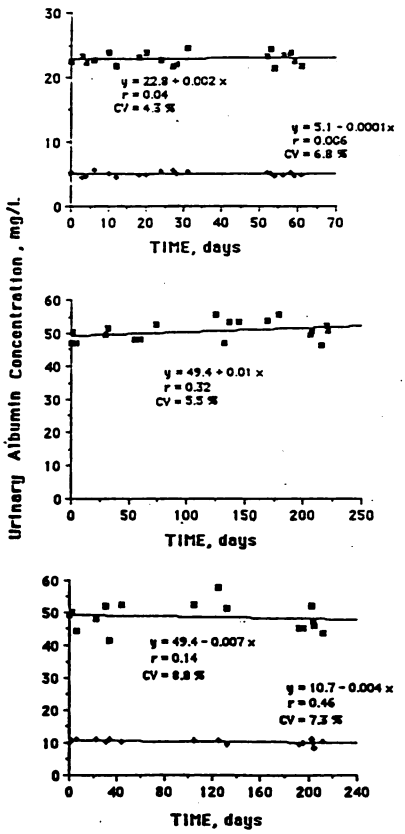

Fig. 1. Time-courses of urinary albumin concentration measured by an RIA kit with a solid-phase system for separation of bound/free phases

Measured albumin values are reported on the *y*-axis, days of storage at −20 °C until assay on the *x*-axis. Between-assay variabilities (CV, %) and the regression analysis for each pool are also shown

ology or, more likely, accuracy of urine collection might play a role. We emphasize that it may be relevant to check the sterility of the urine before assay. In our laboratory, bacterial growth is currently excluded by negativity of urine cultures (*4*). In the studies of Elving et al. (*2*) and Osberg et al. *6*), this precaution was not reported.

Osberg et al. (*6*) reported that the interassay variations of their RIA method were 3.6–7.4%. We wonder how they calculated the interassay variability. How many replicates did they assay? How long was the period of assay? What kind of control samples did they use: urine samples stored at 4 °C or lyophilized material? At what temperature did they store the control samples for their internal quality-control assay? For how long?

An accurate internal quality-control assay is mandatory, because the significant decrease of measured albumin values in stored specimens may be more attributable to changes in the calibration curve of the RIA than to the hypothetical effects of freezing such as conformational changes in urinary proteins, as suggested by Osberg et al. (*6*). We obtained interassay variations quite similar to those reported by Osberg et al. (*6*), using urinary pools stored at −20 °C for as long as 10 months (Figure 1).

In conclusion, in our hands, long-term (up to six months) storage of urine samples at −20 °C does not impair the accuracy of UAE evaluation by radioimmunological methods.

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The authors of the papers referred to respond:

To the Editor:

Giampietro et al. discuss the finding that storage of urine samples at −20 °C lowers the albumin concentra-

tion, as is shown in several other studies (*1–4*). The major difference between the study of Giampietro et al. and the other studies is that Giampietro et al. used pooled urine samples. The decrease in albumin concentration after storage at −20 °C is thought to be due to the formation of a precipitate (*1*). The substance responsible for this precipitation (probably urate) is consequently diluted in the pool. The use of pooled urine can thus mask the decrease of albumin concentration in an individual urine sample. It is therefore impossible to make a proper statement about the effect of storage on albumin concentration when pooled urine samples are studied.

We did not check urine sterility, but in all samples the sediment was studied. Only three of 73 samples showed bacteriuria. These samples were not the ones that showed a decrease in albumin concentration after storage.

Giampietro et al. suggest that a small decrease in albumin concentration is not clinically relevant. We dispute this statement. Patients with slight albuminuria are often studied in therapeutic trials. Minor, but significant differences in albumin concentrations can lead to important conclusions concerning possible reversibility of this albuminuria. It is therefore very important to make an accurate measurement of this analyte.

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To the Editor:

Giampietro et al. report that, in their hands, storing urine samples for six months at −20 °C does not impair the accuracy of measured urinary albumin excretion.

Results for a urine pool are quite different from the results for 101 individual specimens. Not all samples will show the same percentage difference, and in our study, a small number of samples showed no significant decrease on storage. To validate their data, Giampietro et al. should conduct their study with individual samples instead of urine pools. Our study and others have shown a significant decrease in albumin concentration in urine samples stored at −20 °C (*1–4*).

Giampietro et al. also report "significant, but very slight, decrease of albumin concentration . . .in a low-concentration pool; the values of two intermediate-concentration pools did not decrease." We consider this important because the borderline increase in albumin excretion rate (AER), i.e., >95th percentile of healthy normals, may be missed if the urine samples are stored improperly at −20 °C. This stage of slight ("borderline") albuminuria (*5*) may be an earlier predictor of diabetic renal damage. If this is detected early, medical intervention may help prevent the ongoing renal damage.

In our study (*5*), we compared various methods of urine collections; the most consistent were the overnight collections. The 24-h collections tended to be incomplete and were affected by variable degrees of exercise. Urine samples were routinely checked for sterility; if infected, they were cultured and not analyzed for albumin (*6*).

Giampietro et al. reported no decrease in albumin values in four freeze–thaw cycles of 10 urine samples. Ten urine samples is not a large enough number to give any meaningful information. Also, our paper was not addressing the effects of freezing and thawing of the samples.

We believe that freezing the samples may induce conformational changes and thus alter the AER values. Our quality-control results, at two different concentrations, indicate that we have no reason to believe that the decrease of the albumin values seen in our study was due to any changes in the calibration curve of the RIA method. Our interassay CV of 3.6–7.4% was calculated from 100 replicates over six months of lyophilized control material of the same lot number provided in the RIA kits.

If medical intervention will help prevent further damage to the kidney, the "borderline high" values need to be accurately determined. Because AER estimations are becoming routine in patients with diabetes, the number of samples for most laboratories should be adequate to run an assay once every two weeks. If not, the use of reference laboratories should be considered.

We maintain that our report (*4*) was the first evaluation of the effects of freezing on albumin concentrations in urine as determined with a commercial radioimmunoassay kit commonly available in the U.S.

We reiterate that frozen urine samples should not be used for albumin determinations, because freezing may lower the values. This underestimation limits the ability to diagnose borderline albuminuria.

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Dr. Giampietro and colleagues reply: