SHBG and free testosterone measured by the analog assay.

As discussed in our article, adding SHBG by mixing increasing volumes of pregnancy plasma with nondiseased adult male plasma did not reproduce the positive correlation between SHBG and free testosterone, and we found no evidence for SHBG binding of the analog tracer used in the Coat-a-Count kit. Instead, there was a nearly perfect positive correlation ($r = 0.97$) between total and free testosterone among the healthy men we studied. Moreover, free testosterone represented 0.5–0.65% of the total testosterone and did not decrease with increasing concentrations of SHBG, as expected from mathematical calculation and other methods. Thus, we concluded that the analog free-testosterone value provides essentially the same information as does the total-testosterone value.

Drs. Ooi and Donnelly are encouraged to analyze their results in this way. We agree with Drs. Ooi and Donnelly that extensive normative data are essential in both clinical practice and in research studies, but we caution that other methods to determine plasma free testosterone in men should be used to verify the results with the analog free-testosterone assay. A similar concern was raised recently by Rosner (4) in his reanalysis of testosterone concentrations in a study of obese women before and after weight loss.

References


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Editors Note: A correction for Figs. 1 and 3 of the original article by Winters et al. was published on page 445 of the March issue of the Journal.

Multiple Narrow Bands in Urine Protein Electrophoresis

To the Editor:

We studied a 62-year-old man with light chain disease (multiple myeloma) whose urine protein electrophoresis (UPE; electrophoresis performed on agarose gel on a Panagel high resolution electrophoresis system; gel was stained with Amido Black) showed 15 narrow bands in the $\beta$- and $\gamma$-globulin regions; many of the bands were strongly stained and equidistant (Fig. 1), suggestive of a step-ladder pattern originally described for immunofixation electrophoresis (IFE) of urine (1, 2). The patient had blood in his stool, and urinalysis showed microhematuria and a small amount of protein. The abnormal laboratory findings included 23.5 mmol/L urea nitrogen, 848 $\mu$mol/L creatinine, 7.7 mmol/L glucose, 3.11 mmol/L calcium, 72 g/L hemoglobin, a red blood cell count of $2.2 \times 10^{12}$/L, and a hematocrit of 0.20. There was 1.37 g of protein in his urine collected during a 24-h period. UPE showed the pattern just described. Because this was considered similar to the pattern reported after IFE in urine from patients with various conditions (1, 2) and this pattern was reported to be caused by polyclonal light chains (1, 2), a tubular nephropathy was suspected.

A bone marrow aspirate showed no abnormalities (4% plasma cells). Serum protein electrophoresis (Ciba Corning Diagnostic) revealed decreased $\gamma$-globulins, and serum im-

![Fig. 1. Immunelectrophoretic (A) and electrophoretic (B) patterns of urine concentrated 50-fold. (A), a Corning electrophoresis system and antibodies, and Amido Black 10B stain were used. The antibodies in the troughs are, top to bottom: 1, anti-human serum; 2, anti-$\alpha$; and 3, anti-$\lambda$ light chains. Note the anodal distorted arc with anti-$\lambda$ antibodies caused by the presence of $\lambda$-BJP. Anode is at the left. S, control nondiseased serum; P, patient’s urine. (B), electrophoresis was performed with a Panagel system; 45-min run; Amido Black 10B stain. Several bands (farthest cathodal; right) were faint and were seen on the stained agarose plate but are not visible in Fig. 1; anode is at the left.]
muno-electrophoresis (IEP; Ciba Corning) showed decreased IgG, IgA, and IgM and the presence of a faint \(\lambda\) arc with anodal bowing attributable to \(\lambda\)-Bence Jones protein (BJP). Quantification of the serum immunoglobulins (rate nephelometry; QM 300; Beckman) showed 5.17 g/L IgG (reference interval, 7.10–18.58 g/L), 0.62 g/L IgA (reference interval, 0.89–3.87 g/L), and 0.08 g/L IgM (reference interval, 0.46–2.26 g/L). The concentration of \(\beta_2\)-microglobulin in his serum was 16.6 mg/L (reference interval, 1–2.4 mg/L). IEP of his urine (concentrated 50\(\times\); Fig. 1A) showed albumin, a very faint \(\kappa\) arc, and an intense \(\lambda\) arc with bowing at the anodal end (i.e., \(\lambda\)-BJP). The diagnosis of multiple myeloma, light-chain type, was considered, and a bone marrow biopsy performed 1 week later essentially showed replacement of the marrow cells by plasma cells, as seen in multiple myeloma. The first bone marrow aspirate was considered to have been inconclusive because the sample was taken from a region without abnormal plasma cells. Multiple myeloma is often known to have a "spotty" distribution of malignant plasma cells in bone marrow.

Microscopic examination of a kidney biopsy showed a "myeloma kid- ney" pattern, with tubular casts composed of \(\lambda\) light chains (as evidenced by immunofluorescence microscopy). IFE of his urine (concentrated 20\(\times\)) with the Panagel system showed narrow, equidistant bands of \(\lambda\) light chains. However, it was obvious that no anti-\(\lambda\) antibody dilution was optimal for revealing all bands seen in UPE. The utmost importance of the proper antibody concentration in IFE is well known; an excess of antigen can occur and precludes visualization of the intense bands.

The patient underwent routine therapy for multiple myeloma, but his kidney function decreased rapidly; he became oliguric and required chronic hemodialysis. Sixteen months after the findings described above and after therapy, the amount of protein in the patient’s urine decreased substantially and UPE showed fewer (3 to 5) equidistant bands. His serum IFE no longer showed \(\lambda\)-BJP.

Many narrow equidistant bands were seen in the slow \(\beta\) - and \(\gamma\)-globulin regions in high resolution agarose gel electrophoresis of his urine concentrated 10- to 50-fold. Most of the bands in the \(\beta\) - and \(\gamma\)-globulin regions were very intense and sharply defined, others were faint and not equidistant, but all were detected by UPE without the need for immunological enhancement by IFE (Fig. 1B). There also were less-intense bands representing albumin, \(\alpha\)-globulins, and transferrin. After incubation of concentrated (20\(\times\)) urine with 1% 2-mercaptoethanol (2-ME) for 1 h at room temperature, no changes in the electrophoretic pattern occurred. This procedure has been used routinely and successfully in our laboratory to determine the type (light chain) of abnormally appearing IgM in serum IEP. The treatment with 2-ME breaks the polymeric IgM into monomers, thus overcoming the "umbrella effect" often seen with monoclonal IgM. We also incubated concentrated (20\(\times\)) urine with 2-ME overnight at 37°C, after which most of the bands remained the same; however, a few disappeared at the subsequent UPE, and an intense band was seen in the slow \(\gamma\)-globulin region. This suggests that polymers of BJP, which likely were cleaved into monomers after the treatment with 2-ME, were not the sole explanation for the electrophoretic pattern. Different electrical charges of the light chains and their fragments also accounted for the multiple bands seen in UPE.

To our knowledge, such a pattern of UPE from a patient with light chain disease has not been reported. These narrow bands were of \(\lambda\) and not \(\kappa\) type, in contrast to reports on IFE of urine (2), in which \(\lambda\) light chains were found rarely and only together with \(\kappa\) light chains. In two patients with monoclonal gammopathies, the serum M-component was of \(\lambda\) type, but the urine had multiple \(\kappa\)-light chain bands detected by IFE (3). The finding of both \(\kappa\) and \(\lambda\) light chains in the urine of patients with B-cell malignancies is surprising in view of the suppression of normal (polyclonal) immunoglobulins that occurs especially in patients with light chain disease. It should be mentioned that the UPE pattern seen in the patient described above is not common, although we have seen several patients whose UPE had intense, equidistant (albeit fewer) narrow bands. We can only speculate that the \(\lambda\)-BJP from this patient is unusually labile and breaks into fragments and/or forms polymers. The \(\lambda\)-BJP also showed a fainter precipitate with commercial anti-\(\lambda\) antibodies when compared with most of the \(\lambda\) chains we have detected by IEP.

Evenly spaced multiple bands (usually three) were first reported as "the ladder light chain" pattern in urine IFE by Harrison (1). The same pattern, named "urinary light-chain ladder", was later reported by Bailey et al. (3) in IFE of urine from patients with monoclonal gammopathies or other diseases. Although Harrison (2) did not see bands in the UPE (Titan Gel system; Helena Laboratories) because of the stain detection threshold (i.e., the amount of light chain was low), Bailey et al. (3) found a faint banding pattern in the stained agarose gel (in-house system, not commercial reagents). On the basis of two-dimensional electrophoresis and silver staining, Harrison (1) concluded that the pattern he described in IFE of urine can be attributed to polyclonal light chains, even when only \(\kappa\) light chains were detected, i.e., when the bands were apparently monotypic. It is conceivable that the appearance (after protein staining) of equidistant bands that represent light chains in UPE depends on the amount of light chains in the urine and the resolution of the electrophoresis system (e.g., matrix, temperature, and current, as well as the protein stain) (4). It would be interesting to study patients with kidney tubular disease (e.g., Balkan nephropathy) as well as more patients with light chain disease to see whether UPE shows equidistant, multiple bands in the \(\gamma\)-globulin region in these patients.

Multiple bands after electrophoresis of BJP have also been described.


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More on Cystatin C

To the Editor:

I read with interest the letter by Kos et al. (1) on serum cystatin C during malignant progression. This is the first attempt I have seen to explore the increases in cystatin C found in the serum of patients with progressive malignancy. I quite agree with the authors that previous studies have involved too few patients to allow clear-cut conclusions as to whether cystatin C is affected by malignancy.

The authors have shown that there is a progressive increase in the relative amount of cystatin C (compared with their healthy controls) in patients with primary metastatic melanoma and in patients with colorectal cancer. Unfortunately, interpretation of their data is complex. There are common misunderstandings that a lack of change in serum creatinine implies a lack of change in glomerular filtration rate (GFR) and that it is essential that cystatin C correlate exactly with serum creatinine.

Kos et al. (1) have not presented the creatinine data that go with their cystatin C results, so it is difficult to know whether creatinine was increased in (some of) the patients with increased cystatin C. The most important point is that they have not used a reference technique to measure GFR to establish whether, in patients with progressively more serious malignancy, there was any change in GFR.

The studies I have been involved in (2, 3), and those of other groups (3, 4), have clearly shown that cystatin C is a more sensitive indicator of changes in GFR than creatinine. Indeed, cystatin C can be increased in situations where there are no apparent changes in serum creatinine. In view of the very small changes in cystatin C reported, less than two-fold proportional increases, I feel it is essential that independent assessment of GFR is performed to establish whether the increases in cystatin C are attributable to increased production rate or to decreased elimination by glomerular filtration.

The data showing that in malignancy there are increases in the mRNA for cystatin and cysteine proteinases do provide some evidence that there will be an increased production or increased release of cystatin C into the serum of such patients. When performing a study such as Kos et al. (1) describe that intends to establish whether there is an increased concentration in the serum of patients, it is inappropriate to use serum creatinine as an indicator of a lack of change in GFR. The increase in serum cystatin C in colorectal cancer and metastatic melanoma may indicate the influence of nonrenal factors on the concentration of cystatin C. However, in the absence of a reference GFR procedure to eliminate any changes in clearance, this work acts more as a stimulant to further study rather than as definitive evidence.