High Incidence of False-Positive Albuminuria Results with the Micro-Bumintest™, Mark Colwell1 and John F. Halsey2,3 (1 IBT Reference Lab, 6811 W. 63rd St., Overland Park, KS 66202, and 2 Dept. of Biochem. and Molec. Biol., School of Med., Univ. of Kansas, Kansas City, KS 66103)

To monitor renal integrity in diabetics, one tries to detect abnormal urinary albumin excretion at its earliest stages ("microalbuminuria"), e.g., when albumin concentrations in urine are about 30 mg/L, below the limit of conventional dipstick sensitivity (1, 2). Miles Laboratories (Ames Division, Elkhart, IN) has introduced the Micro-Bumintest™ for use in screening urines for albumin, 40–300 mg/L. We compared the results of this test with albumin values determined by a radioimmunoassay (Diagnostic Products Corp., Los Angeles, CA).

Urine specimens from patients being routinely evaluated in two different endocrinology clinics were assayed in duplicate for albumin concentration by RIA, then further tested with the Multistix® SSG reagent strips (Miles Laboratories, Ames Division). The Multistix was used to measure pH, relative density, presence of blood, and other sources of interference of the Micro-Bumintest. Of the 103 urines tested, 85 had albumin concentrations <30 mg/L by RIA and were the focus of further consideration. The Micro-Bumintest gave positive test reactions for 79% (87/85) of these (Table 1), but there were numerous false positives, 45/62 (73%), even if we used a lower cutoff value, 17 mg/L. Thresholds of both 30 and 17 mg/L have been recommended by various investigators (3, 4). A cutoff of 30 mg/L reportedly gives very high specificity, because no individual without renal disease would be expected to have a urinary albumin concentration >30 mg/L (3). Others (4) have recommended the higher sensitivity provided by a 17 mg/L threshold.

The package insert mentions several possible explanations for falsely positive results: high pH, high relative density (specific gravity), and the presence of detergents or skin cleansers. None of these would explain our results. The control samples provided with the RIA kit were within the specified ranges. No standards or controls are provided with the Micro-Bumintest. A clarified normal urine without detectable albumin, fortified with albumin to give known concentrations, performed satisfactorily in both tests. In these cases, urines with <30 mg/L albumin score negative on the Micro-Bumintest. The urine specimens were stored in the refrigerator and assayed within the time frames recommended by the manufacturers. Apparently, nonprotein components in urine interfere with the bromphenol blue reaction (5) that is the basis of this test. Unless a correction can be made for these interfering substances, we cannot recommend use of Micro-Bumintest in screening diabetics.

References


Radioimmunoassay of thyrotropin (TSH) is well-established, and highly sensitive radioimmunoassay is often used clinically (1). Some recent results indicate that TSH immunoreactivity and bioactivity are not always synonymous (2). The detection limit of present bioassays for bovine TSH is about 0.5–1 milli-int. unit/L (3–6). More-sensitive bioassay is necessary for evaluating human TSH.

The diterpene forskolin, which activates hormone-sensitive adenylate cyclase by acting directly at the catalytic subunit of enzyme, potentiates hormonal agonists that act through GTP-binding protein (Ga) (7). Low concentrations of forskolin also potentiate the cAMP response of thyroid cells to TSH (7, 8); the detection limit of an assay for bovine TSH is about 10 milli-int. units/L (8). Here, we used this diterpene in our bioassay involving FRTL-5 cells (6) to develop an ultrasensitive bioassay of TSH.

Simultaneously adding 0.1 μmol of forskolin per liter with TSH potentiated the cAMP response of thyroid cells to TSH, the response with forskolin being parallel to that without it (Figure 1).
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References


Fig. 1. Effect of forskolin on FRTL-5 cell cAMP response to bovine thyrotropin or human chorionadotropin (hCG). Cultures were exposed to increasing doses of thyrotropin or hCG together with forskolin, 0.1 μmol/L (○); forskolin, 0.5 μmol/L ((); or in Hank’s balanced salt solution containing 0.5 mmol of 3-isobutyl-1-methylxanthine and 4 g of bovine serum albumin per liter alone (C). All incubations were for 2 h. Bars indicate the mean ± SD for each set of triplicate cultures subjected to each treatment.

Whereas the response of cAMP to TSH alone (1 milli-int. unit/L) was 7.6-fold that of the control culture without TSH, including forskolin (0.1 μmol/L) in the test incubation increased the cAMP response by 31-fold. Comparable potentiation was observed for all doses of TSH tested. This assay can thus detect as little as 0.1 milli-int. unit of TSH per liter, much less than any previous bioassay (3–6). Similarly, use of a higher concentration (0.5 μmol/L) of forskolin increased the response of cAMP, but the basal cAMP was also increased (Figure 1). Therefore, inclusion of 0.1 μmol of forskolin per liter suffices for an ultrasensitive bioassay.

We applied this ultrasensitive assay to measure the thyrotropic activity of human chorionadotropin (hCG), to try to resolve conflicting reports about its thyrotropic activity (9, 10). The cAMP increase was noticeably stimulated with as little as 15 kilo-int. units of crude hCG (Mochida Pharmaceutical Co., Tokyo, Japan) per liter in the presence of 0.1 μmol of forskolin per liter, whereas 63 kilo-int. units/L was required in the absence of forskolin (Figure 1).

This ultrasensitive bioassay may be useful for detecting thyrotropic activity of low concentrations of human TSH and hCG as well as thyroid-stimulating autoantibodies.

References

Stability of Sex-Hormone-Binding Globulin in Serum and Plasma, Gernot Sinnecker (Dept. of Pediatrics, Univ. of Hamburg Eppendorf, Martinistr. 52, 2000 Hamburg 20, F.R.G.)

Measurement of sex-hormone-binding globulin (SHBG) in serum is of clinical significance in several pathological states, including hirsutism (1), androgenetic alopecia (2), and androgen insensitivity syndromes (3). Several immunological methods for the measurement of SHBG are currently used in research and for clinical purposes (4–6). However, little is known about the immunological stability of this protein in serum and plasma samples. I have systematically examined the stability of its immunoreactivity in human serum and plasma, using a radioimmunoassay as earlier described (4).

Specimens of serum and plasma from four male and four female patients were stored at 4 °C, at room temperature, and at 37 °C. Aliquots were removed for assay at appropriate intervals and stored at −20 °C until assayed. Four samples underwent repeated freeze–thaw cycles—storage at −20 °C, followed by thawing at room temperature for 10 min, and then refreezing at −20 °C—as many as 30 times. Aliquots of the thawed material were removed and stored at −20 °C until assayed. To make sure that the initial freezing of the sample did not alter the value, I measured fresh and once-frozen aliquots of four samples on the same day that the blood was drawn. SHBG was measured by RIA in quadruplicate, in two different dilutions. All aliquots of one sample were run in a single assay. The data for serum and plasma were separately analyzed.

No changes in radioimmunologically detectable SHBG concentrations were observed after storage at 4 °C or at room temperature for up to eight weeks. In contrast, storage at 37 °C caused a relatively rapid loss of immunoreactive SHBG. However, even these samples remained stable for three days (Figure 1). Repeated freeze–thaw cycles caused no loss of the immunologically measurable SHBG, nor did the initial freezing of the sample alter the value. Results were the same for serum and plasma.

Although purified SHBG is known to be relatively unstable (4), its immunoreactivity remained fairly stable in whole serum and plasma. I conclude that the time between specimen collection and assay is not crucial in processing blood samples for the RIA of SHBG. Repeated measure-