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Direct Radioimmunoassay of Melatonin in Saliva

To the Editor:
Investigations into use of the pineal gland hormone, melatonin, as a putative biological marker for the effective disorders (1, 2) and for pinealoma (3, 4) have generally relied on results from radioimmunoassay. The advent of sensitive, specific methods for estimating melatonin in plasma has done much to facilitate clinical research, but there are practical difficulties associated with taking samples of blood at midnight or early morning for melatonin assay. The current assay for concentration of hormones in saliva (6), which obviates such difficulties, has led to our developing such an assay for melatonin that involves no extraction with solvents, which—with use of a new tracer with high specific activity—we have used in investigating the relationship between plasma and saliva (Miles et al., in preparation).

We used N-(tris(hydroxymethyl)methyl)glycine ("Tricine"), activated charcoal (untreated powder), Dextran T70, and melatonin from Sigma Chemical Co., St. Louis, MO 63178; [3H]melatonin (spec. act., 31 kCi/mmol) from Ameraham International plc, Amerham, Bucks, U.K.; antisera (batch no. 704/6483) from Guildhay Anti sera, University of Surrey, U.K. All other commonly used laboratory reagents were of "Analyzer" grade, from British Drug Houses, Poole, Dorset, U.K. Disposable plastic apparatus was used wherever possible and all glassware was acid-washed and rinsed in methanol before use.

Saliva, sampled without stimulation of its production, was collected into disposable plastic tubes and stored at -20 °C for at least 24 h. After thawing, the samples were centrifuged at 2500 × g for 15 min to remove solids. We assayed 500-μL duplicate aliquots, comparing results directly against a standard curve prepared in assay buffer (0.1 mol/L "Tricine," pH 7.4, containing 9 g of NaCl and 1 g of gelatin per liter). The standard curve covers the range 0 to 100 pg per tube. We used the sequence 0, 2.5, 5.0, 12.5, 25.0, 50.0, and 100 pg per tube.

The procedure: To 500 μL of saliva and standard add 150 μL of antisera (initial dilution, 2000-fold in assay buffer). Vortex-mix the contents of the tubes and incubate them at room temperature for 4 min. Then add 50 μL of buffer containing 3000 cpm of [3H]melatonin to all tubes, vortex-mix, and incubate at 4 °C for 20 h. Perform all subsequent procedures at 4 °C. Separate the antibody-bound melatonin from the free fraction by incubating the mixture with 450 μL of dextran-coated charcoal suspension (10 g of charcoal, 0.1 g of Dextran T70 per 500 mL of assay buffer, stirring the mixture at 4 °C) for 10 min. Then centrifuge for 10 min at 1700 × g. Transfer 600 μL of the supernate to 4 mL of toluene-based scintillant, shake the mixture for at least 30 min, and equilibrate in a scintillation counter before counting the radioactivity. Using the standard curve, calculate the melatonin concentrations in the samples. Within- and between-assay CVs for 12 replicate standard curves showed the precision of the assay system to be satisfactory (Table 1). Analytical recovery of hormone, measured at three different concentrations, ranged from 97.9 to 103.5% with an assay sensitivity (defined as the least amount of hormone distinguishable from zero) of 4 pg per tube. Nonspecific binding of antibody was <2%. The antibody characteristics have been characterized by the University of Surrey, U.K. The only measurable cross reactivity (expressed relative to the quantity of melatonin that replaces 50% antibody-bound [3H]melatonin) was found to be from N-acetyltryptamine (0.91%), 6-hydroxymelatonin (0.33%), and N-acetyltryptophan (0.22%). All other structurally related compounds that we tested cross reacted by <0.06%.

In a recent study of the nocturnal profile of salivary melatonin (Miles et al., in preparation) we have shown that the secretory pattern of melatonin in plasma is mirrored in saliva, except that the mean concentration is 24% that in the corresponding plasma. Results for paired plasma and saliva samples, taken hourly from six healthy volunteer subjects between 20:00 and 08:00 hours, correlated very well (r = 0.971; p < 0.001). Hormone secretion in these volunteers was greatest at 01:00 hours, the melatonin concentration in plasma being 94.8 ng/mL (SEM 15.3) and the corresponding value for saliva being 24.4 ng/mL (SEM 3.1) at 08:00 hours, the concentration in plasma had declined to 33.9 ng/mL (SEM 5.9) with 9.7 ng/mL (SEM 1.8) being detectable in saliva. Melatonin reportedly is about 70% bound to plasma albumin, and the salivary fraction may thus represent the "free" (i.e., unbound) hormone. Differences in the plasma—saliva ratio for melatonin seen at some points in compared overnight plasma and salivary hormone profiles were suggested by results of a subsequent study to be ascribable to a dilution effect, i.e., changes in salivary flow rate. Such differences were minimized if saliva was collected without any stimulation of its production.

We anticipate that assay of melato-
nin in saliva analysis may prove to be useful as an adjunct to its assay in plasma where information about the biologically available hormone is required, or as an alternative to plasma analysis when venepuncture is inconvenient, impracticable, or undesired.

We thank Dr. K. Callender for her valued interest and advice, Mr. R. Vaughan-Williams for his helpful criticism, and Mr. Gofam M. S. Lewis for his encouragement.

References

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Breast Milk or Not? A Clinical Conundrum

To the Editor:

Harrison's Chemical Methods in Clinical Medicine (1) includes a section on "conundrums," describing how interesting clinical problems may be solved by the use of simple chemical tests. We describe one such problem and our solution to it.

A 21-year-old West Indian lady was delivered of a 29 weeks' gestation infant but was initially unable to produce milk from the breast. The baby was therefore fed on expressed mother's milk from a milk bank. Being a vegetarian, the mother very reluctantly allowed the infant to be fed on cows' milk formula. After two months, the mother claimed to be producing her own milk and brought a sample to our special-care baby unit. It was thought

that she might be providing samples of soya milk, claiming it was her own.

Remembering that human breast milk has very high γ-glutamyltransferase (EC 2.3.2.2.) activity (2), we measured the activity of this enzyme in a sample of the claimed mother's milk, in control samples of milk known to be human breast milk, and in a soya-formula milk. The respective activities were 634, 461, and 85 U/L. These results provided good presumptive support for the mother's claim. Indeed, the patient was subsequently observed to produce her own milk.

References

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Pitfall in Treatment of Anion-Exchange Resin before Total Iron-Binding Capacity in Serum is Measured

To the Editor:

Total iron-binding capacity (TIBC) of serum has principally been measured by using either sodium bicarbonate or anion-exchange resin to remove free iron, after saturating the transferrin in the sample with iron. The latter method was originally reported by Peter et al. (1), who stated that "the resin (Amberlite IRA 410) should be washed with HCl and buffer solution, and then should be dried at 95 °C." When we followed their method, the dried resin particles became white (from brown); when they were added to buffer solution the mixture became viscous, and a "popping" sound was heard.

Light microscopy showed the presence of cracks on the surface of resin IRA 410 particles when we dried them at 95 °C, and their spherical shape was distorted when they were mixed with buffer solution. There was less change if the resin particles were dried in the air at ambient temperature on filter paper. These effects were not observed for resin IRA 402, the stronger basic anion-exchange resin, which is said to be stable to heating.

Values for TIBC of commercial calf serum shown in Table 1 were measured by the method of Peter et al. (1) except for use of various resins (IRA 410 dried at 95 °C, IRA 410 dried at room temperature, and IRA 402 dried at 95 °C). Higher values for TIBC were obtained when resin IRA 402 was used. The maximum amounts of Fe citrate adsorbed onto resins 410 and 402, dried at 95 °C, were respectively 24 and 4.0 µg per 300 mg of dry resin. Evidently, resin IRA 402 dried at 95 °C may not completely remove free iron, after saturating transferrin in the serum, even though this resin is stable to heating.

Generally speaking, exposure of the resin to high temperature is undesirable. In this case, however, the heating method caused the change on the surface of the resin IRA 410 particles such that it increased their ability to adsorb free iron. Peter et al. did not mention this characteristic of IRA 410. It is interesting that this unexpected "pitfall" has played an important role in the clinical use of this method.

Reference

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Use of Lithium to Determine Volume of 24-h Urine Specimens

To the Editor:

Because information on the amounts of substances excreted in the urine per day is often diagnostically and prognostically important, 24-h urine specimens are often collected in clinical investigations, metabolic balance studies, and research. However, well-known difficulties attend their accu-