where $A_i$ is integrated absorbance and [Cd] is in $\mu$g/L. The standard errors of the intercept were 0.002 and 0.003 and the standard errors of the slope were 0.004 and 0.006 for the calibration and standard addition curves, respectively. The calibration and standard addition slopes are statistically different, with $F$- and $t$-tests confidence levels of 99.5% [16]. Therefore, matrix effect is important and standard addition graphs must be used.

We studied the limit of detection (LOD = 3 SD/m) and the limit of quantification (LOQ = 10 SD/m), where SD is the standard deviation of 11 measurements of a blank and m is the slope of the standard addition graph. The LOD and LOQ were 22.5 and 74.9 $\mu$g/kg, respectively.

The within-run precision ($n = 11$) CVs for low, normal, and above-normal concentrations were 6.9%, 4.2% and 2.5%, respectively. The intraassay CV for four determinations was 3.8% at a cadmium concentration of 0.08 $\mu$g/g. The total SD (slurry preparation and ETAAS measurement) calculated from 11 different slurries prepared from the same hair sample, the cadmium concentration being determined in each slurry twice over 2 weeks, was 6.9%. The analytical recoveries ($n = 11$) of cadmium added to the hair slurry at concentrations of 0.25, 0.50, and 1.00 $\mu$g/L were 101.0% ± 3.3%, 102.0% ± 2.4%, and 100.0% ± 1.4%, respectively. For CRM 397 human hair Reference Material with a certified cadmium content of 0.521 ± 0.024 $\mu$g/g, we obtained a 99.5% confidence interval of 0.533 ± 0.015 $\mu$g/g (mean ± s SD/√n), where $n = 11$ determinations and $t$ is the Student's t-value [16] for $n - 1 = 10$ degrees of freedom. To study the influence of the slurry concentration on the precision and analytical recovery of the method, we prepared slurries that contained 2, 3, 4, 8, and 12 g/L hair, corresponding to 0.05, 0.075, 0.1, 0.2, and 0.4 g of sample, respectively. The CVs ($n = 11$) were <2.5% for all slurry concentrations and the analytical recoveries were 97.0% ± 3.1% to 103.0% ± 2.3% for all slurry concentrations and for the two cadmium concentrations tested (0.5 and 1.0 $\mu$g/L).

We found no interfences from the following species at concentrations of 1 mg/L: Ag $^{+}$, Al $^{3+}$, As $^{3+}$, Ba $^{2+}$, Ca $^{2+}$, Co $^{2+}$, Cr $^{3+}$, Cu $^{2+}$, Fe $^{3+}$, K $^{+}$, Mg $^{2+}$, Mn $^{2+}$, Ni $^{2+}$, Pb $^{2+}$, Sc $^{3+}$, Sn $^{4+}$, Sr $^{2+}$, Zn $^{2+}$, Br $^{-}$, Cl $^{-}$, I $^{-}$, SO$_{4}^{2-}$, and SO$_{3}^{-}$.

In 13 scalp hair samples, the cadmium concentrations were 0.022-0.19 $\mu$g/g. Reported cadmium values were 0.3 $\mu$g/g for healthy people from Europe (Poland and Sweden) [3, 17, 18], 0.5-1.5 $\mu$g/g for North Americans (Canada and US) [17, 19], and 0.3 $\mu$g/g for Japanese [17]. The cadmium content in human hair is related to food intake. Study of the possible correlation between cadmium in hair and in other samples such as blood and urine is needed [3].


References

globulins that react with both the H and the M subunits and bind LD1-LD5; and (c) immunoglobulins that react with isoenzymes that contain both the H and the M subunits and bind LD2-LD4. As an example of one of the latter cases, Kanemitsu [5] postulated the existence of an immunoglobulin that reacts with a trimer consisting of two H and one M subunits.

In this study, the patient had an extremely high LD activity with an abnormal LD isoenzyme pattern, which was induced by the binding of specific IgA with LD5 and, partially, LD4 isoenzymes. We investigated the specific IgA and propose a cause of the marked increase of LD activity in the patient's serum.

A 9-year-old boy presented with fever and was admitted to a nearby hospital. Some of the relevant laboratory test results (and reference intervals) for serum analysis are: white blood cell 11.7 \(\times 10^9/\text{L}\) (4-9 \(\times 10^9/\text{L}\)), C-reactive protein 87.0 mg/L (0.0-3.0 mg/L), aspartate aminotransferase 25 U/L (11-40 U/L), alanine aminotransferase 13 U/L (7-45 U/L), and LD 2 610 U/L (238-463 U/L). The patient was diagnosed as having acute tonsillitis, and antibiotic treatment was initiated. After 2 days, the fever abated and C-reactive protein was on the decrease. However, LD activity was still high (\(~2500\ U/L\)). The patient was admitted to Nagano Children's Hospital for further examination. The increased LD activity persisted through 8 months of observation.

LD activities were determined with a commercially available kit. In our laboratory, the reference interval for healthy subjects is 125–215 U/L. Electrophoresis [7] and immunofixation [10] were carried out on agarose gel in barbital buffer, pH 8.6. Blood cells (erythrocytes and lymphocytes) were prepared by the method described previously [11].

The patient's LD isoenzyme pattern was characterized by a broad band around the region of the LD4 isoenzyme, with a normal mobility of LD1, LD2, and LD3 and an apparent absence of LD5 (Fig. 1A). The patient's erythrocytic and leukocytic LD isoenzyme patterns were normal. The presence of LD-IgA(A) complex in the patient's serum, which is more uncommon than the LD-IgA(\(\alpha\)) complex [12], was demonstrated by the immunofixation method.

Ion-exchange chromatography with DEAE-Sephalcel was performed for partial purification of the patient's IgA. The patient's partially purified IgA was mixed with an equal volume of the control serum, which consisted predominantly of either LD2 and LD3 isoenzymes or LD4 and LD5 isoenzymes. The mixtures were electrophoresed and developed for LD bands (Fig. 1B). The original patterns of the control sera with nearly equal LD activities were altered to the variants (with the broad band in the region of LD4 and the absence of LD5). In both cases, however, the intensities of the broad bands were quite different. No change was observed in the mobility or the intensity of LD1, LD2, and LD3 before or after the mixing in either case. The intensities of abnormal bands in the region of LD4 depended on the respective LD5 bands of the original control sera. Mixing the appropriate dilutions of the patient's partially purified IgA with normal serum produced a phased change in the pattern of normal LD5 isoenzyme to that of the variant, whereas the patterns and the intensities of LD1, LD2, and LD3 isoenzymes were almost constant.

Sera were appropriately mixed to get the LD pattern with a similar intensity for all of the five isoenzymes. The mixed sera were subjected to electrophoresis and reacted with the patient's partially purified IgA, followed by fixation with anti-IgA antibody.

![Electrophoretic LD isoenzyme patterns.](image)

(A) The patient's serum (A-1), and a hemolysate of the patient's red blood cells (A-2) and white blood cells (A-3). (B) The control serum, consisting predominantly of either LD2 and LD3 isoenzymes (B-1) or LD4 and LD5 isoenzymes (B-2), mixed with an equal volume of saline (a) or the patient's partially purified IgA (b). LD bands were developed after electrophoresis of the mixtures. (C) Control serum in duplicate. One lane (C-1) was directly stained for LD isoenzymes and another lane (C-2) was reacted with the patient's partially purified IgA followed by fixation with anti-IgA antibody, with subsequent staining for LD isoenzymes.

Body and development of the LD bands (Fig. 1C). Only LD4 and LD5 isoenzymes were observed after the fixation, and the ratio of LD5/LD4 changed to 9.87 (from that of the original mixed sera, 1.70). The affinity of LD5 against the specific IgA was about six times higher than that of LD4. This indicates that the specific IgA recognizes a tetramer of the M subunit and, weakly, a trimer of it. In other words, the specific IgA preferentially reacts with the particular peptide sequences made from an adjacency of the four and, partially, three M subunits and not with the individual M subunits.

The specific IgA bound to LD could be a monoclonal antibody, for the following two reasons: (a) The light-chain restriction (\(\lambda\) light chain) supports the possibility of a monoclonal response; and (b) the blotting assay of the patient's partially purified IgA involving hog muscle LD5 as a ligand showed a sharp LD band compared with the broad band of the whole IgA. The patient's IgA neither competed with the substrate and coenzyme nor inhibited LD activity. This could suggest that the binding site of the specific IgA does not include the region from which activity arises (the NAD and substrate binding sites) or a site causing a change of tertiary structure related to inactivation.

The stability of LD activity was compared between the
patient's serum and the control serum of a patient by storage at 5°C for 26 days. No significant change was observed in the LD activity of the patient's serum, whereas the control serum decreased during the investigation by 27%, from 2148 to 1578 U/L.

The markedly high activity of serum LD is explained by the high concentrations of LD5 accumulating in the patient's serum due to the delayed catabolism of LD-IgA complexes. The half-life of LD5, ~9 h [13], which is the shortest among the five LD isoenzymes, was prolonged to nearly a week, the half-life of IgA [14], by the formation of LD5-IgA complexes. In previous reports, LD activity levels in the serum of patients with LD-IgA(α) complexes were <1000 U/L [1, 3, 4]. In most of these cases, the LD isoenzymes participating in the LD-IgA complexes were mainly LD3 and to a lesser degree LD2, whose half-lives are approximately 31 and 75 h [13], respectively. The participation of the LD5 isoenzyme in the complexes could be the main reason for the markedly high level of LD activity in this patient, compared with those of the cases previously reported. The deviation of LD, including the LD5 isoenzyme from many kinds of cells, in this patient is probably at a normal level, as in other cases. The increased stability of LD activity according to the formation of the complexes, which was confirmed by a storage test, could also be another reason.

To our knowledge, ours is the first report of a complex between IgA and LD5 and, partially, LD4 isoenzymes but not LD1, LD2, and LD3, confirmed by experimental evidence.

6β-Hydroxycortisol Interferes with Immunoassay of Urinary Free Cortisol

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Urinary free cortisol (UFC) is considered a more reliable index of adrenocortical hyperfunction than is total serum cortisol. About 1% of the total urinary steroid ordinarily appears as free (non-protein-bound) cortisol [1, 2]. When the total serum cortisol concentration exceeds the saturating concentration of cortisol-binding globulin (CBG), then the concentration of free cortisol found in the urine is increased. Thus, the concentration of UFC reflects the serum concentration of free cortisol, the fraction considered to be biologically active [3, 4]. Measurement of UFC is of clinical significance particularly for the signs and symptoms of adrenocortical hyperfunction (e.g., Cushing syndrome) and after corticosteroid stimulation tests. The most common method of determining UFC concentrations in the clinical laboratory is immunoassay, either enzyme immunoassay (EIA) or radioimmunoassay (RIA), the specificity of which depends largely on the characteristics of the antibody in the analytical system.

6β-Hydroxycortisol (6β-OHF) is closely related to cortisol in molecular structure (see illustration). A polar metabolite of cortisol, 6β-OHF is formed by cytochrome P450 3A enzyme (CYP3A) exclusively in the endoplasmic reticulum of hepatocytes [5, 6]. This monoxygenase enzyme is easily inducible by common drugs such as antipyrene, phenobarbital, rifampicin, or phenytoin and concomitantly produces an increased excretion of urinary 6β-OHF [7–9]. Although high amounts of urinary 6β-OHF would be expected to interfere with measurement of UFC concentrations by immunoassay, no information on such a problem is yet available. Here we report the cross-reactivity of 6β-OHF and the nature of its interference in analyses of UFC concentrations by EIA or RIA.

6β-OHF was purchased from Steraloids, Wilton, NH. For an analysis of UFC concentration, we purchased three EIA and three RIA kits from five different commercial vendors. Assay procedures were followed as instructed by the manufacturers.

We calculated cross-reactivity by the method of Nieschlag and Wickings [10]: % cross-reactivity = a/b × 100, where a/b is the ratio of the cortisol concentration (a) to the concentration of 6β-OHF (b) that replaces 50% of the labeled cortisol.

To assess interference, we used the multiple regression model [11], y (A,l) = β0 + β4A + β5l + β6A·l, where A is the concentration of analyte (cortisol), l the concentration of interferent (e.g., 6β-OHF), A·l the term for analyte–interferent interaction, and β the various coefficients. The multiple regression expression was derived from a 4 × 4 matrix composed of four different concentrations of cortisol (0, 20, 50, and 150 μg/L) and

References


Cortisol

6β-Hydroxycortisol