decision threshold to "rule out" an infarction, i.e., using a test with a high sensitivity (θ), alters with time after infarction in such a manner that, for any chosen decision threshold, test sensitivity increases, then decreases, with time (I). Thus, up to 6 h after an infarction, a decision threshold of 7 U/L for CK-2 gives a test sensitivity of 95%, whereas 13–18 h after infarction, the sensitivity becomes 99% (I). Consequently, the conventional reference ranges shown in Table 1 imply test sensitivities in excess of 90%, except during the first 6 h after infarction. To "rule in" an infarction, i.e., by using a test with a high specificity (ε), requires a decision threshold for CK-2 (U/L) of between 15 and 23 U/L, depending on the level of specificity needed; this threshold does not alter with time after infarction (I). Evidently, the conventional decision threshold is too low to be useful for "rule in" purposes.

We also examined the incidence of abnormal total CK activities in our population. In 15% of all cases, the first abnormal result (in terms of CK-2, defined as in Table 1) had normal total CK activity, i.e., ≤174 (males) or ≤140 U/L (females). However, the following sample always had an abnormal (as defined above) total CK activity.

As with CK-2, the "rule out" decision threshold for total CK also varies with time (I). Thus, 7–12 h after infarction, a threshold of 90 U/L gives a 95% sensitivity, whereas after 13–18 h the test has a 99% sensitivity. Others have also shown that a normal total CK activity does not necessarily rule out infarction (9).

In uncomplicated myocardial infarction, therefore, a substantial number of patients may show an incomplete spectrum of CK abnormalities in the first abnormal specimen. For example, we have already shown (I) that the CK-2 (%) measurement is more often positive at an earlier stage than the CK-2 (U/L) assay. Now we also show that the "or" and "and" diagnostic rules are nearly equivalent for these CK-2 assays. Accordingly, enzymatic criteria for myocardial infarction must take account of the evolving nature of the serum enzyme patterns. Of course, it is also necessary to reiterate what we have previously demonstrated, as have others before us, that decision thresholds change in the course of a myocardial infarction (I): such a situation further complicates the interpretation of serum enzyme data.

References

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Novel IgA Antibody to Bovine Thyrotropin in a Healthy Man

To the Editor:

Antibodies to thyrotropin (TSH) receptor (TRAB) are thought to be responsible for hyperthyroidism of Graves' disease and also to be a pathogenic factor in primary atrrophic hypothyroidism (I). A radioreceptor assay for detection of the antibody (TSH-binding inhibitor immunoglobulin, TBI) has been developed by Shewring and Smith (2), and is widely used as a commercial kit (Baxter Co., Tokyo, Japan) (3). However, TBI is measured as abnormally low when test serum contains substances that bind to bovine TSH (4). In a study of 75 healthy staff volunteers, we found a case with abnormally low concentration of TBI caused by a novel IgA-type antibody to bovine TSH.

A 36-year-old man was discovered to have abnormally low TBI (19% below normal), expressed as percent inhibition of <sup>125</sup>I-labeled bovine TSH binding to TSH receptor (3). His family history was uninformative, and there was no family history of thyroid disease. Physical examination was normal, and he did not have goiter. Values in tests related to thyroid function were in normal ranges, and tests for thyroid microsomal and thyroglobulin autoantibodies both gave negative results.

The TBI result in this case was 7.5 SD below the normal mean (0.4%, SD 2.6%, n = 74), and nonspecific binding of labeled bovine TSH (bTSH), measured by substituting 10 mM/L L-idubol (polyethylene glycol monododecyl ether) solution for the TSH receptor, was 29.7% (normal mean 8.6%, SD 0.8%, n = 11). The binding of labeled bTSH to serum sample was inhibited in a dose-dependent manner by the addition of unlabeled bTSH, but not by human TSH. Moreover, in the in vitro TSH bioassay system with FRTL-5 cells (5), the immunoglobulin fraction significantly inhibited bTSH-induced cAMP increase (69% inhibition compared with that in the presence of normal immunoglobulin fraction) but did not inhibit human TSH-induced increases.

To identify the immunoglobulin class of this TSH-binding substance, we incubated the IgG fraction with <sup>125</sup>I-labeled bTSH and counted the radioactivity of the immunoprecipitate after adding anti-IgG antibody. The radioactivity was only slightly higher (10.9%) than normal range (7.9% ± 0.5%, mean ± SD, n = 11). To determine the molecular size of the binding substance, we fractionated the serum sample on a Sephacryl S-300 column. The binding of <sup>125</sup>I-labeled TSH to each fraction was estimated by adding polyethylene glycol (150 g/L, final concentration) and counting the radioactivity of the precipitate after centrifugation. A single peak of the radioactivity was found in the fraction with the approximate molecular mass of 230 kDa. Moreover, this peak of the radioactivity disappeared after an immunoadsorption of the serum sample with anti-IgA antibody. Autoradiogra-
Fig. 1. Autoradiography of 125I-labeled bTSH binding of serum samples after immunoelectrophoresis for anti-human immunoglobulins. Immunoelectrophoresis of serum samples (upper serum from this subject; lower, control human serum) was performed by using anti-human whole serum antibody (a,b), anti-human IgG antibody (c), and anti-human IgA antibody (d). After the immunoelectrophoresis, the specimens were stained with Brilliant Blue R (a), or subjected to 125I-labeled bTSH binding study (b,c,d).

phy of 125I-labeled bTSH binding of the serum sample after immunoelectrophoresis for anti-human immunoglobulins revealed that the radioactivity migrated with the electrophoretic IgA precipitin line with slower mobility (Figure 1).

These studies suggest that this bTSH-binding protein in the healthy subject is an IgA-type antibody to bTSH.

The existence of antibody to TSH has been reported in patients with autoimmune thyroid diseases (4, 6, 7), especially in those with Graves' disease, but it is rarely observed in healthy subjects (8). The TSH antibodies reported previously were autoantibody (6, 7) or heteroantibody (4, 8), and were all in the IgG class (4, 6-8). However, there has been no report of IgA-type TSH antibody.

In autoimmune thyroid diseases, thyroid microsomal and thyroglobulin antibodies of IgA class have been reported (9). Moreover, Leino et al. (10) reported the increase in IgA class antibodies against Yersinia enterocolitica in these diseases, and suggested a causal relationship between infection with this pathogen and the diseases. However, ours is the first report of an IgA-type TSH antibody in a normal healthy man. Because this subject had never received bTSH injection, we consider this antibody to be spuriously produced. The mechanism of the production of this IgA-type antibody, including genetic factors, is to be elucidated further.

References


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Direct Analysis of Capillary Blood Glucose with Kodak Ektachem Analyzers

To the Editor:

Until the middle of May 1990, we analyzed glucose either in venous blood (collected with sodium heparin and sodium fluoride) or in capillary blood, the latter accounting for nearly 40% of all samples. We mixed 50 μL of the sample with 750 μL of a diluent containing 150 mmol of NaCl, 48 mmol of NaF, and 1 mmol of potassium EDTA per liter, and centrifuged at 500 × g for 5 min. Glucose in the supernate was analyzed by a method based on glucose oxidase coupled to the Trinder reaction (GOD-PAP, cat. no. 100 4746; Boehringer-Mannheim, Mannheim, F.R.G.; (1)), applied to the Greiner G-300 analyzer (Greiner Electronics AG, Langenthal, Switzerland).

The method with the G-300 analyzer had been validated years ago by comparison with a method based on glucose dehydrogenase (2). Recently, we acquired Ektachem 700 XRC analyzers equipped with a PC II probeoscis (Eastman Kodak Co., Rochester, NY). The accompanying slide method for glucose is also based on a glucose oxidase/Trinder reaction but can be used only with serum, plasma, cerebrospinal fluid, or urine (3). We have compared the slide method for plasma with our routine method, and modified the sample collection procedure for capillary blood to enable direct analysis with the Ektachem systems.

Venous blood samples collected with sodium heparin and sodium fluoride were used in this study. Glucose was analyzed by four methods:

(A) A 50-μL aliquot of whole blood was mixed with 750 μL of the diluent prepped into 5-mL plastic tubes (cat. no. 55 475; Sarstedt, Nurnbrect, F.R.G.) for analysis with the Greiner G-300 analyzer, as described above.

(B) A 75-μL aliquot of whole blood was mixed with an equal volume of the diluent in a 1.5-mL Eppendorf tube supplied with a cap (no. 72 690; Sarstedt); this tube was placed inside the plastic tube used in procedure A. The diameters of the two tubes ensured that the inner Eppendorf tube remained at the tip of the outer plastic tube when centrifuged at 500 × g for 5 min. The cap of the Eppendorf tube was cut off and the plastic tube containing this inner tube was loaded on the sample quadrant of the Ektachem analyzer. Glucose was analyzed by using the test channel ordinarily reserved for urinary glucose, and the results were multiplied by two.

The concentration of NaF in the sample-diluent mixture in this procedure is lower than in the original method (24 vs 44.8 mmol/L). To test if this lower concentration of fluoride was sufficient to inhibit glycosylation, we diluted four samples containing different amounts of glucose with equal