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## Reliability of Kit Methods for Free Thyroxin: The Corning Test System

*To the Editor:*

In regard to the recent profusion of commercial radioimmunoassay (RIA) test kits for free thyroxin (FT4), a thorough consideration of the applied methodological principle is essential for clinical evaluation of the assay results. Lawlor and Blaustein (*1*) compared FT4 measurements as performed with two commonly used test kits, the Clinical Assay Gamma Coat FT4 kit and the Corning Immophase FT4 Test System, concluding that the latter may give unreliable results in the presence of various non-thyroidal diseases. The Corning Test System has a rather unconventional assay procedure, and, although widely discussed, the relation between the variables quantified and the FT4 concentration in the sample does not seem to be clear in this technical approach (*2, 3*).

Two independent measurements are made in the Corning Test. In the first step, $^{125}$I-labeled T4 tracer solution containing a T4-displacing agent is used. As is usual in RIA procedures, the percentage of antibody-bound activity ("series B") indicates the total T4 (TT4) concentration. In the second measurement, which is performed in the same way with "pure" radioligand, the percentage of bound tracer ("series A") is related to the concentration of FT4. Both results, which are claimed to represent the two points of a kinetic determination, yield an absolute FT4 concentration value by calculation of the product A X TT4 concentration and use of a calibration curve.

In the absence of a T4-displacing agent, binding of FT4 to antibody surely would be functionally related to the FT4 concentration in the serum sample. At constant antibody capacity, the added tracer will bind correspondingly and theoretically can give a measure of the original FT4 value. But considering the decrease of bound activity of only 6 to 8% over the whole measuring range of the calibration curve (FT4 concentration range 5–60 ng/L), the functional relation between FT4 concentration and bound radioligand is quite insufficient under these conditions. A further interpretation for the slight decrease in bound activities at increasing FT4 concentrations must be taken into account. The extent of tracer binding observed can be a result of the distribution between antibody and specimen plasma proteins, in particular thyroxin-binding globulin (TBG), and with consideration of the tracer concentration used, the extent of binding as determined by the amount of FT4 appears to be completely negligible. So far, the decrease in antibody-bound activities with increasing FT4 values takes place *only* if the TBG concentrations increase simultaneously. Conversely, at low concentrations of TBG, high amounts of bound activity would be found regardless of the concentration of FT4 in the sample.

We have performed a simple experiment which seems to confirm this assumption (*4*): In a dilution series of different samples with *decreasing* TBG and T4 concentrations, but (approximately) *unchanged* FT4 values (produced by mixing pregnancy serum with different amounts of serum from a euthyroid patient with hereditary TBG-deficiency), the Corning Test System yielded apparently increasing FT4 values at TBG values <15 mg/L (normal range 13–27 mg/L) (*3*). At 5 mg of TBG per liter, an apparent FT4 concentration of about twice the normal value for this series was measured.

These findings suggest that the bound activities counted as "series A" represent a (reciprocal) measure for the TBG concentrations. Therefore, by calculating the product A X TT4 concentration the Corning Test reflects the TT4/TBG ratio, but not the FT4 concentration as such. In logical consequence, correlation between the Corning Test and the TT4/TBG ratio has been found to be excellent (*5*). This restriction should be considered in the clinical evaluation of Corning FT4 measurements in sera containing concentrations of TBG significantly different from normal.


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## Valproic Acid Does Not Interfere with an Enzymatic Determination of Free Fatty Acids in Plasma

*To the Editor:*

In a recent Letter (*1*), we showed that the antiepileptic drug valproic acid (VPA) interferes significantly with colorimetry (*2*) of free fatty acids (FFA) in plasma. We suggested the use of a more specific procedure for determination of FFA in plasma of subjects who are being treated with VPA.

Determination of FFA in the presence of VPA may be needed both in epileptic patients and in pharmacological studies dealing with binding of VPA to plasma proteins. In fact, FFA modulate VPA plasma protein binding, both in vitro (*3*) and in vivo (*4*). So we decided to see if another simple procedure for routine determination of FFA (the ACS-ACOD enzymatic method, *5*) is subject to the same interference. Briefly, the analysis is based on two coupled enzymatic reactions, with formation of a final colored product, which is measured at 550 nm.

The apparent concentrations of FFA (expressed as oleic acid) determined in a pure aqueous solution of VPA, 700 μmol/L, in drug-free plasma, and in the same plasma to which VPA (700 μmol/L) was added, were respectively 2 ± 1, 660 ± 20, and 660 ± 22 μmol/L (mean ± SD, n = 10).

Evidently VPA does not interfere, so this method can be an alternative to gas-chromatographic methods for determination of FFA in plasma samples containing VPA.


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## Marked Post-Operative Increase in Serum Aspartate Aminotransferase Activity

*To the Editor:*

Causes of hepatocellular damage in the immediate post-operative period include drugs, hypoxemia, sepsis, and decreased vascular perfusion of the liver (*1, 2*). In the typical patient with hepatocellular dysfunction after surgery, the range of aspartate aminotransferase (AST; EC 2.6.1.1.) activity is two- to 10-fold the upper limit of normal (*2*). In a small minority of these patients, there is massive hepatic necrosis. However, AST activity exceeding 5000 U/L is extremely unusual and, if present, is usually accompanied by hyperbilirubinemia (*3*).

A patient is presented who in the immediate post-operative period had an AST value of >8000 U/L, which was not accompanied by hyperbilirubinemia. The hepatic cell damage was accompanied by pre-renal azotemia, suggesting that decreased perfusion of the liver and kidney was responsible for organ damage.

A 72-year-old diabetic man was admitted to the emergency department complaining of chest pain. His temperature was 38 °C, pulse rate 120/min, and blood pressure 180/100 mmHg. The left foot had an infected abscess. Investigations done to determine the cause of chest pain included an electrocardiogram and creatine kinase assay; results of both were normal. Two days after admission, with the patient under general anesthesia, the abscess was incised and drained. Thirty-six hours after surgery, the patient complained of chest pain. An electrocardiogram was again normal, as was creatine kinase. However, the activities of AST and lactate dehydrogenase (EC 1.1.1.27.) were markedly above normal (Table 1).

The patient denied any exposure to hepatoxins or to individuals with hepatitis. He had not received any blood products. A test for hepatitis B surface antigen was negative. Total serum bilirubin concentration was normal. The patient was also anuric at this time and was dehydrated. He was treated with intravenous fluids. Table 1 shows the resulting decrease in values for both liver enzymes, urea nitrogen, and creatinine.

On the basis of the patient's history and the time course of the increased AST, it is extremely unlikely that viral hepatitis or hepatotoxins such as halothane were responsible for the marked increase in AST; the most probable cause was diminished blood flow to the liver. This conclusion is supported by the fact that the patient also had prerenal azotemia, which also reflects poor circulatory status (*4*). Moreover, renal function improved and liver enzymes decreased simultaneously with repletion of fluid, which lends further support to this hypothesis.

The most important reason for recognizing the occurrence of markedly increased AST values secondary to fluid depletion is to distinguish the condition from viral or toxic hepatitis. If AST increases because of a poor circulatory state, its activity can be expected to decline rapidly with improvement in circulatory status (*5, 6*). In viral or drug-induced hepatitis, high AST activity persists longer, with no relationship to the circulatory status of the patient.

**Table 1. Serial Changes in Values for Aspartate Aminotransferase (AST), Lactate Dehydrogenase (LD), Urea Nitrogen, and Creatinine (CR) in Serum, before and after Surgery**

| Hours before (−) and after surgery | AST, U/L (19–38)[a] | LD, U/L (90–210) | Urea N, mg/L (50–130) | CR, mg/L (7–12) |
|---|---|---|---|---|
| −24 | 32 | 117 | 120 | 9 |
| 24 | — | — | 200 | 20 |
| 36 | >8000 | 7420 | 620 | 28 |
| 46 | 6860 | 3920 | 340 | 24 |
| 72 | 3400 | 1360 | 300 | 17 |
| 96 | 1050 | 427 | 200 | 12 |
| 180 | 36 | 166 | — | — |

[a] Normal range in parentheses.

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## Is There an Enzymatic Reversibility of Nonenzymatic Glycosylation of Hemoglobin?

*To the Editor:*

Recently, evidence has accumulated that the biochemical basis of some diabetic complications involves nonenzymatic glycosylation of certain long-lived proteins (*1*). Glucose molecules react with amino groups of proteins, forming primarily an aldimine, the Schiff base, which is subsequently stabilized after Amadori rearrangement as a covalent ketoamine adduct (*2–4*). Hemoglobin, for example, is converted to hemoglobin $A_{Ic}$. However, no data on the metabolism of the hemoglobin–glucose adduct are available as yet. Our object was to look for an enzymatic activity cleaving the protein–monosaccharide bond.

$HbA_{Ic}$ was isolated, purified, and characterized from blood of human diabetics by the method of Bunn et al. (*5*), and 0.1-mL samples containing 1 mg of $HbA_{Ic}$ were digested with enzymes and enzyme preparations as listed in Table