

The discrepancy between the testosterone concentrations with and without extraction was further investigated. We deduced that the serum could not contain excess testosterone bound to some unknown or modified binding protein, as all protein-bound testosterone should have been extracted with the ether. This point was further elaborated by extracting the sample after acid hydrolysis before assay, again giving <0.5 nmol/L. Accordingly, we assayed for testosterone conjugates that might be present. Serum proteins were precipitated with ethanol, aliquots of the supernatant evaporated, redissolved in water, and either treated with sulfatase or acid hydrolysis to obtain testosterone free of sulfate or other conjugates such as glucuronide. The conjugate-free testosterone was extracted with ether and assayed in the Spectria Testosterone ^{125}I kit. Again <0.5 nmol/L testosterone was found, proving that testosterone conjugates were not present in inordinate amounts in the serum.

The possible presence of antibodies in the patient's serum was further investigated, although we had deduced that these antibodies could not be directed against testosterone as such. However, if the serum contained binding substances for ^{125}I testosterone, this would also lead to falsely high concentrations in the assay, as less indicator would bind to the solid phase. Accordingly we incubated serum with ^{125}I testosterone overnight at 4°C and precipitated the IgGs in the sample with protein A-Sepharose (Zymed Laboratories, South San Francisco, CA). Since 83% of the added ^{125}I testosterone was recovered in the protein A-Sepharose precipitate vs 5% in a control serum, the sample contained an IgG that bound ^{125}I testosterone. To prove that the IgG was specific for ^{125}I testosterone, we incubated with ^3H testosterone and precipitated with protein A-Sepharose as before. Only 0.8% of the added ^3H testosterone was recovered with the protein A-Sepharose precipitate, showing clearly that the patient's serum contained an antibody that bound the ^{125}I testosterone of the Spectria Testosterone ^{125}I kit, but without any activity against testosterone as such.

The ^{125}I testosterone binding properties of the patient's serum were evaluated by incubating the serum with different concentrations of ^{125}I testosterone overnight at 4°C and precipitating the IgGs in the sample with protein A-Sepharose. After determining the spe-

cific activity of the tracer by comparing the tracer displacement of different doses of ^{125}I testosterone with the testosterone calibrator of the Spectria Testosterone ^{125}I kit, the binding data were subjected to Scatchard analyses with the IBM PC program Radlig (Biosoft, Cambridge, UK). One binding site with $K_D = 5.2$ (0.5) nmol/L and $B_{\text{max}} = 4.8$ (0.1) nmol/L [mean (SE)] was found in the patient's serum.

Patient 2: A woman, 45 years old, moderate hirsutism, regular periods, low concentrations of sex hormone binding protein (SHBG) (18 nmol/L), slightly increased concentrations of androstendione, other examined hormone concentrations normal except for testosterone at >50 nmol/L. The serum was reassayed for testosterone after diethylether extraction, giving 1.2 nmol/L. This serum was tested for ^{125}I testosterone binding as for patient 1. One binding site with $K_D = 0.10$ (0.03) nmol/L and $B_{\text{max}} = 4.3$ (1.0) nmol/L [mean (SE)] was found in the patient's serum.

Both patients were at risk of receiving the wrong diagnosis because of high serum concentrations of testosterone. Patient 1 had in fact already undergone one laparotomy largely on the basis of falsely high concentrations of testosterone. Thus the presence of ^{125}I testosterone antiserum in the patient's blood is not only a laboratory problem, but clearly a diagnostic and therapeutic problem, too.

The prevalence of antibodies against ^{125}I testosterone is difficult to assess. We detected two patients in 2 months (1 in 800 samples). These sera were detected because of extreme testosterone serum concentrations, and we have no information on whether moderately increased testosterone samples may contain ^{125}I testosterone antibodies. Likewise we do not know whether the ^{125}I testosterone of other direct testosterone kits may react against endogenous antibodies. The testosterone tracer from Orion contained testosterone-19- ^{125}I histamine. We have not tested whether the antibodies bind to ^{125}I testosterone labeled in other ways, or to histamine. Although we do not know the specificity of the antibodies (except that they do not bind testosterone), our observations are potentially important because testosterone-19- ^{125}I histamine is a widely used indicator in testosterone immunoassays. We hope this letter will lead other laboratories to carefully evaluate high testos-

one concentrations in women and re-assay samples after extraction when in doubt.

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Clinical Utility of Carbohydrate-Deficient Transferrin to Detect Alcohol Abuse in a General Population

To the Editor:

An isoform of transferrin, carbohydrate-deficient transferrin (CDT), has been found to be increased in serum in a high percentage of alcoholics [1, 2]. CDT measures an accumulated effect of alcohol consumption, appearing after regular intake of 50–80 g of ethanol per day over several weeks. Decreased glycosylation of transferrin protein before hepatocellular release is the most likely mechanism for the production of CDT. After separation of the different isoforms of transferrin by (e.g.) isoelectric focusing, chromatofocusing, or anion-exchange chromatography, CDT can be quantified by means of binding-assays [1, 2]. We evaluated the clinical utility of a commercially available kit (CDTectTM) from Pharmacia (Uppsala, Sweden), which is based on the technique of Stibler et al. [3].

We studied 138 volunteers. Details about alcohol consumption were obtained by a confidential self-report wherein the total amount of alcohol intake (daily number of beverages) during the last 4 weeks was reported. The control group (group 1) consisted of 24 healthy male volunteers with a mean daily alcohol consumption <5 g during the last 4 weeks before blood sampling. Group 2 consisted of 42 clinical patients (18 women and 24 men) with various alcoholic (cirrhosis, fatty liver) and non-alcoholic liver diseases (chronic hepatitis, primary biliary cirrhosis, toxic fatty liver, cholangitis). According to self-report and an interview with one member of their family, they had been abstaining for 1–6 months before blood sampling. Group 3 consisted of 72 men who were regular pub visitors. They were interviewed and

sampled in a pub during the "happy hour," late one Friday afternoon. According to the self-interviews, 53 subjects in the pub had a regular mean daily alcohol intake of 35 g (range 0–45 g) (group 3a); the 19 other subjects reported a daily alcohol consumption of at least 100 g during the last month (group 3b). Procedures followed were in accordance with the policies of the Institutional Review Board of the Hospital Group (ECOM).

All blood samples were analyzed for γ -glutamyltransferase (GGT) and mean corpuscular volume of erythrocytes (MCV) and by CDTECT. Serum GGT was determined according to IFCC recommendations at 30 °C with a Hitachi 717 analyzer (Boehringer Mannheim, Mannheim, Germany), and MCV of erythrocytes was determined with a HITM analyzer (Technicon, Tarrytown, NY). Isoforms of transferrin were separated by micro anion-exchange column chromatography and measured with a double-antibody solid-phase RIA. Intraassay precision (CV), based on 33 measurements, was 6.2% and 4.9% for CDT values of 8–12 and 30–35 U/L, respectively. The interassay precision (CV) was 10% at a mean value of 31 U/L.

As Table 1 shows, all subjects in group 1 had serum CDT concentrations below the upper normal limit of the manufacturer's reference interval (20 U/L for men and 25 U/L for women). Mean CDT, GGT, and MCV values were 12.9 (range: 8.3–19.4) U/L, 11.0 (3–21) U/L, and 92 (88–97) fL, respectively.

Serum concentrations of CDT in 38 of the group 2 subjects (abstinent subjects with liver diseases) were below the upper normal limit: mean 12.1 U/L [11.0 (7–18) U/L for the men and 14.2 (5–19) U/L for the women]. However, another

four of these abstinent patients (two men with liver cirrhosis, a woman with chronic hepatitis, and a woman with non-alcoholic fatty liver) had substantially increased CDT.

Eight of 19 heavy drinkers in group 3b had increased CDT: mean (range) 49.1 (21.4–187.2, $n = 8$) U/L; in the other 11, mean CDT concentrations were 11.8 (9.3–15.4) U/L. Although the GGT value of group 3 as a whole was much higher [70.0 (8–239) U/L] than for control group 1 [11.0 (3–21) U/L], there was no correlation between CDT and GGT values; mean GGT values were 52.0 (8–161) and 83.8 (15–239) U/L in the CDT-positive and -negative subjects, respectively. In addition, in group 3a, among 53 self-declared social drinkers, only 1 subject had a high CDT concentration (CDT 32.2 U/L, GGT 8 U/L, MCV 91 fL). The mean (range) serum CDT for the other 52 subjects was 11.6 (6.2–17.2) U/L, with an increased mean GGT [59.9 (4–1122) U/L]. In the whole group 3, the CDT measurement had a sensitivity of 42.1%, which is below the sensitivity of the GGT assay (47.4%) in our study.

By receiver-operating characteristic (ROC) curve analysis, even when we compared the data for the heavy drinkers (group 3b) with those for the control subjects (groups 1 and 2), the sensitivity of the CDT assay was below that of GGT at false-positive rates of <50%. The areas under the ROC curves for CDT and GGT were 0.67 and 0.76 with 95% confidence intervals of 0.52–0.81 and 0.62–0.90, respectively—indicating that these tests were essentially useless in this setting.

The clinical performance of the CDT

measurement varies according to the population studied. According to Stibler [1], a sensitivity of 82% and a specificity of 97% can be achieved by CDT measurements in an alcoholic population. On the other hand, in a population examined at a general medical practice, in which the subjects display a wide range of alcohol consumption, the diagnostic sensitivity decreased to $\pm 45\%$ [4, 5]. The utility of CDT measurements in discriminating between occasional social drinkers and alcoholics in a general population has not been reported. In our study the analytical performance and the diagnostic specificity of the CDTECT assay were acceptable. However, the diagnostic sensitivity of this assay is not sufficient for use as a screening test in a general population.

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Table 1. CDTECT results in three groups of subjects categorized according to alcohol use.

Group	No. of subjects	Alcohol consumption, g/day ^a	Assay cutoff values		
			CDTECT, ≥ 20 U/L	GGT, ≥ 45 U/L	MCV, ≥ 101 fL
1, volunteers (abstainers)	24	0–5	0	0	0
2, liver disease	42	0	4	39	1
3, pub group a	53	0–45	1	8	3
b	19	>100	8	9	1

^a During the previous 4 weeks according to the self-interviews; ranges are shown.

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An author of one of the works referred to replies:

To the Editor:

In their Letter, Vermes and van den Bergh address a currently popular issue. They describe three groups they studied: