Two patients with low T_{3} by the Amerlex-M method, and the "sick euthyroid syndrome," had normal values for T_{3} by the Amerlite method. The misclassified patients were older than 50 years and were receiving different treatments, depending on the nature of the presenting disease. The discrepancies were not due to noticeably icteric, turbid, or hemolyzed samples. The small size of the study does not permit extensive evaluation of the diagnostic efficiency of the Amerlite kit. The single patient identified as hyperthyroid was correctly classified by both methods, T_{3} values being \( > 4 \) nmol/L. The present study indicates the need for further investigation before introduction of the Amerlite T_{3} assay into a routine laboratory.

I. Ramasamy
Dept. of Biochem.
Prince Charles Hospital
Merthyr Tydfil
South Wales, U.K.

Oligoclonal Bands and Post-\( \gamma \)-Globulin in Electrophoretograms of Cerebrospinal Fluid

To the Editor:
Assessment of cerebrospinal fluid (CSF) for oligoclonal immunoglobulin banding has become an important part of the diagnostic evaluation of patients with multiple sclerosis and other neurological disorders. Successful band detection depends on the selection of proper electrophoretic and staining techniques and on experience in interpretation of the electrophoretic patterns. Currently, high-resolution electrophoresis on agarose gel, followed by staining with Coomassie Brilliant Blue or silver, is the most commonly used method for detection of oligoclonal bands in CSF. Other methods such as isoelectric focusing in polyacrylamide and agarose gels or detection by immunofixation with specific antisera have been used much less frequently, mainly because they are more time-consuming and require greater technical skills.

Several problems can be encountered in the process of oligoclonal band detection: deterioration of oligoclonal banding owing to proteolytic degradation during storage of CSF; insufficient volume of CSF for the concentration of the sample; or failure to detect oligoclonal bands due to low immunoglobulin concentration (1). In addition, one or more non-immunoglobulin bands can be detected in the region of oligoclonal band migration (2–5); probably the most prominent of these is post-\( \gamma \)-globulin. A "unique protein" of non-immunoglobulin nature migrating in the middle of the \( \gamma \)-globulin region of normal CSF electrophoretograms (6) and the occasional presence of an artefactual band in the same position (2, 3) have also been reported. In this report we would like to focus on post-\( \gamma \)-globulin; in our experience, it is frequently present in CSF electrophoretic patterns and it should be recognized in the interpretation of oligoclonal bands.

Post-\( \gamma \)-globulin—also known as \( \gamma \)-trace protein, \( \gamma \)-globulin, or cystatin C (preferred designation)—is a low-molecular-mass basic protein with cysteine proteinase inhibitory activity. The relatively high concentration of this protein in CSF (7.3 \( \pm \) 2.4 mg/L, mean \( \pm \) SD) and mean CSF-blood concentration ratio of 5.2 suggest its local synthesis within the central nervous system (7). Post-\( \gamma \)-globulin was isolated and partly characterized physicochemically in our laboratory (8), and its concentration was measured in CSF of patients with various neurological disorders in an attempt to establish its clinical significance. As shown in Figure 1 (pattern 1), post-\( \gamma \)-globulin can be detected as a homogeneous, fast cathodically migrating band on agarose gel electrophoresis (Panagel; Princeton Separations, Adelphia, NJ 07710). We have observed a similar pattern with discrete post-\( \gamma \)-globulin band in about 20% of CSF samples sent to us for oligoclonal band detection, using concentrated (with Coomassie Blue staining) or unconcentrated (with silver staining) CSF. The identity of post-\( \gamma \)-globulin was determined by immunofixation (9) with a specific anti-post-\( \gamma \)-globulin antisera (Figure 1, pattern 2) raised in rabbits (8).

Although the molecular mass of post-\( \gamma \)-globulin is rather low (13 260 Da), it is retained in the concentrate on ultrafiltration through a Minicon CS15 concentrator (Amicon Corp., Lexington, MA 02173), the equipment commonly used to concentrate CSF before electrophoresis. If it is present in a sufficient concentration, post-\( \gamma \)-globulin is readily detectable in the electrophoretic pattern and should not be interpreted as one of the oligoclonal bands. To prove the presence of true oligoclonal immunoglobulin bands, the agarose electrophoresis should be repeated and supplemented by immunofixation, as already suggested by others (3–5). No post-\( \gamma \)-globulin antisera is commercially available now, but antisera specific for immunoglobulin heavy chains can be used for this purpose. Alternatively, isoelectric focusing in polyacrylamide or agarose gel can be used—a procedure that well resolves post-\( \gamma \)-globulin from immunoglobulin oligoclonal bands (3).

The effect on the mobility of post-\( \gamma \)-globulin of storage, even at temperatures as low as \(-20^\circ\) C, has been reported by several authors (3, 8). After storage of CSF for two weeks or longer, the protein assumes higher anodic mobility and can be detected as an additional narrow band close to the point of application (Figure 1, pattern 3). Therefore, in certain situations two bands can be detected in the electrophoretogram. This phenomenon should also be taken into consideration in the interpretation of CSF oligoclonal bands.

References

Jan Čejka
Dept. of Lab. Medicine
Children's Hosp. of Michigan
Detroit, MI 48201

Karel Kithier
Dept. of Pathology
Wayne State Univ. School of Med.
Detroit, MI 48201

Relationship between Fructosamine and Plasma Lipid Concentrations in Patients with Diabetes Mellitus

To the Editor:

We have proposed an automated colorimetric assay for glycerated serum proteins (fructosamines) based on the reducing activity of whole serum in alkaline solution (1). Fructosamine results paralleled HbA1c values and provided an index of blood-glucose control over the previous one to three weeks in patients with diabetes mellitus (2). However, in vitro studies suggested significant interference by lipemia in the method (1).

Abnormalities of plasma lipid concentration are common in diabetes mellitus, particularly among obese, non-insulin-dependent diabetic individuals (3). The primary biochemical abnormality is an increase in very-low-density lipoprotein concentration accompanied by a decrease in high-density lipoprotein cholesterol. Values for low-density lipoprotein cholesterol are usually within normal limits. These changes, which manifest as an increase in plasma triglyceride, and to a lesser extent an increase in plasma total cholesterol concentration, are exacerbated by poor metabolic control, relative insulin deficiency, and inappropriate dietary management.

Because 80% of the diabetic population is non-insulin-dependent (4), any interference in the fructosamine assay by lipemia would seriously limit the clinical usefulness of the test. Here, we investigated the extent of interference from hypertriglyceridemia in vivo by comparing serum fructosamine and HbA1c concentrations in 37 patients with insulin-dependent diabetes mellitus (IDDM), 52 patients with non-insulin-dependent diabetes mellitus (NIDDM), and 41 healthy volunteers. The HbA1c assay was performed after washing erythrocytes to eliminate interference in the assay from lipemic plasma. Diagnosis of diabetes was based on criteria proposed by the National Diabetes Data Group (4). All participants had normal liver and renal function and a normal hematologic profile.

Fructosamine concentrations in samples of serum collected before meals were determined by commercial methods in a Cobas Bio centrifugal analyzer (F. Hoffmann-La Roche, Basle, Switzerland). HbA1c proportions were measured by isoelectric focusing and laser densitometry (LKB, Bromma, Sweden). Triglyceride concentrations were measured by a commercial enzymic method (Boehringer Mannheim, F.R.G.) in plasma sampled after an overnight fast.

A high proportion of diabetic patients had significantly increased results for fructosamine and HbA1c, consistent with mediocre to poor blood-glucose control (2). Triglyceride concentrations during fasting ranged from 0.47 to 2.0 (mean 3.19) mmol/L in NIDDM patients, from 0.51 to 2.84 (mean 1.07) mmol/L in IDDM individuals, and from 0.46 to 5.23 (mean 1.05) mmol/L in non-diabetic controls. Twenty-four (46.2%) NIDDM patients, three (8.1%) IDDM patients, and one (2.4%) non-diabetic control had hypertriglyceridemia (fasting plasma triglyceride >2 mmol/L) and eight patients had severe hypertriglyceridemia (fasting triglyceride >5 mmol/L).

Nevertheless, fructosamine concentrations were closely correlated with HbA1c values for non-diabetic and diabetic individuals, including the eight patients with severe hypertriglyceridemia (Figure 1). Moreover, there was no trend of increasing interference with increasing triglyceride concentration despite grossly high triglyceride concentrations in some of the patients.

We conclude that measurement of fructosamine in whole serum provides a valid index of blood-glucose control, even among diabetic individuals with moderately severe hypertriglyceridemia.

References

John Baker
Charles Small
Roger Johnson
Green Lane Hospital
Private Bag, Symonds Street
Auckland 1, NZ

Effect of Digoxin-like Immunoreactive Factor on the TDx Digoxin II Assay

To the Editor:

We read with interest the report of Gault et al. (1) concerning the response of the Abbott TDx Digoxin II assay to digoxin-like immunoreactive factor (DLIF). Reports of the presence of DLIF in serum of neonates (2), and of hepatic failure (3) and renal-failure patients (4,5), prompted us to investigate similar groups of patients' samples for DLIF interference with the TDx Digoxin II assay. Above-normal values for bilirubin and creatinine were used as indicators of hepatic and renal impairment,