routine testing when 10 specimens that immediately followed specimens with high ALT and (or) AST activities were re-assayed with the SMAC and the ABA-100. The data summarized in Table 2 support the acceptable correlation discussed above between the SMAC and ABA-100, and further highlight the extent of the carryover problem. The carryover characteristics of both enzymes were very similar.

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Simplified Immunonephelometric Quantitation of Apolipoprotein B in Hyperlipoproteinemic Serum

Claus C. Heuck and Günther Schlierf

An immunonephelometric procedure for determination of apolipoprotein B in human serum [Clin. Chem. 25, 221-226 (1979)] was modified by using the detergent octadecenylamine-polyoxyethylene, which may bind selectively to the phospholipid moiety of lipoproteins. Preincubation of hyperlipemic serum samples with lipases could then be omitted.

Additional Keyphrases: atherosclerosis • lipoproteins • lipases • heart disease

Having observed that lipophilic cations show a high affinity for very-low-density lipoproteins (VLDL) (1), we studied the effect of various types of detergent on light-scattering development during immunoreaction with apolipoprotein B. We found that alkylamine derivatives of polyoxyethylene, unlike other nonionic detergents at concentrations below the critical micellar concentration of detergent, bind selectively to VLDL but not to low-density lipoproteins (LDL), causing a decrease in nonspecific light-scattering from immunocomplexes with VLDL. With this modification, we could obviate the enzymic pretreatment that we reported recently for nephelometry of apolipoprotein B in serum (2).

Materials and Methods

General Procedures

Serum samples from normolipemic and hyperlipoproteinemic subjects were analyzed routinely as previously described (2). The very-low-density and low-density lipoproteins were isolated by ultracentrifugation (3). Apolipoprotein B was quantitatively measured by radial immunodiffusion with commercially available agarose plates (Partigen; Behring-

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Fig. 1. Maximum light scattering during immunocomplex formation of anti-apolipoprotein B with isolated VLDL (below) and isolated LDL (above) in presence of octadecenylamine-polyoxyethylene (A), n-octylphenyl-polyoxyethylene (B), or isodi-}

cel1-polyoxyethylene (C)

Final detergent concentrations are given on the abscissa. These data were obtained after 3 h of immunoreaction at 23 °C

werke, Marburg, G.F.R.) in serum and in subnatant fractions of serum samples ultracentrifuged at density 1.006 g/L as previously described.

Light-Scattering Measurements

We measured the development of light scattering during immunocomplex formation between anti-apolipoprotein B (batch No. 4901 a; Behringwerke)\(^1\) and isolated VLDL or LDL with a low-angle scattering laser nephelometer (Behringwerke), as described (2).

Effect of various detergent concentrations. We made qualitative measurements at various concentrations of detergent over a period of 3 h at 23 °C. The following detergents—generously presented by Dr. Krapf (BASF, Ludwigshafen, G.F.R.)—were used: octadecenylamine-polyoxyethylene (I), n-octylphenyl-polyoxyethylene (II), and isodi-}
cel1-polyoxyethylene (III). VLDL or LDL were diluted 51-fold (20 μL of lipoprotein solution with 1 mL of solvent) with either isotonic saline or isotonic saline containing various concentrations of detergent. To 100 μL of this diluted lipoprotein we added 200 μL of antiserum that had been appropriately diluted with isotonic saline (2).

Effect of phospholipase C treatment on immunonephelometry in the presence of detergent I. VLDL or LDL, diluted 51-fold with isotonic saline or with a solution of detergent I (200 mg/L), were preincubated with phospholipase C (EC

3.1.4.3; Boehringer, Mannheim, G.F.R.) (90 kU/L of diluted VLDL) at 23 °C for 30 min. To 100 μL of this solution was added 200 μL of diluted antiserum. For comparison, untreated VLDL or LDL was diluted with either isotonic saline or detergent solution before addition of antiserum. Light scattering was measured for 3 h at 23 °C. Detergents II and III (data not given in figures) were similarly tested.

Effect of phospholipase A\(_2\) treatment on the immunonephelometry in presence of detergent I. A similar experiment was made with phospholipase A\(_2\) (EC 3.1.1.4; Boehringer) for enzymic lipolysis (90 kU/L of diluted VLDL). The experiment was conducted in essentially the same way as described above.

Quantitative measurements. Ten microliters of fresh serum or subnatant fraction of serum samples ultracentrifuged at density 1.006 g/L was diluted with 1 mL of a solution of detergent I (200 mg/L). Two-hundred microliters of antiserum diluted sixfold with isotonic saline was added to 100 μL of the diluted samples and gently mixed. Light scattering was measured as described previously (2).

Analytical Variables

Precision. In a series of 20 aliquots, samples of a serum with type IV hyperlipoproteinemia (triglyceride concn., 2.69 g/L; cholesterol concn., 2.57 g/L), the coefficient of variation (CV) for within-batch precision was 4.3%. The day-to-day CV was 5.3% as measured during 13 consecutive days with use of a serum sample with type IIa hyperlipoproteinemia (triglyceride concn., 0.82 g/L; cholesterol concn., 3.06 g/L).

Linearity. The linearity of the standard curve was estimated by use of a series of dilutions of a commercially available standard serum (β-lipoprotein standard, Behringwerke).

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\(^1\) This antiserum, raised against a subfraction of isolated LDL, is commercially available. By the double immunodiffusion technique, we found no precipitin lines with apolipoproteins A\(_1\), A\(_2\), C\(_1\)–\(_3\), or with high-density lipoprotein.
The log-log standard curve was linear within the range 40 to 500 mg of apolipoprotein B per liter (2).

**Results and Discussion**

Maximum reduction of light scattering during the formation of immunocomplexes between native VLDL and apolipoprotein B develops at lower concentrations with detergent I in the mixture than with detergents II or III (Figure 1). At higher concentrations, however, the maximum reduction is the same for all three of these surfactants. At low detergent concentrations the differences may be attributed to a higher affinity of detergent I for the surface of VLDL. Detergent I does not change light scattering in the immunoreaction with LDL, whereas detergents II and III both reduce the maximum light scattering at increasing concentrations. Obviously detergents II and III interact with LDL, whereas detergent I does not bind to LDL sufficiently to affect its immunonephelometric characteristics.

To evaluate a specific site at which detergent I binds to the lipoprotein surface, we preincubated VLDL and LDL with various lipases. Incubation of VLDL with phospholipase C in the absence of detergent caused an increase in light scattering during the immunoreaction, probably because of decreased hydration of the lipoprotein surface (Figure 2). However, VLDL pretreated with phospholipase C in the presence of detergent I showed no decrease in light scattering as had been observed with native lipoprotein. With detergent II and III, light scattering was decreased even after enzymic preincubation.

The effect was similar after pretreatment of VLDL with phospholipase A₂ (Figure 3). The typical decrease in light scattering during immunocomplex formation developed only with native VLDL in the presence of detergent I; it did not develop after enzymic lipolysis. Light scattering was also diminished with detergent III but not with detergent II after phospholipase A₂ lipolysis.

On pretreatment of LDL with both phospholipase C and phospholipase A₂ in the presence of detergent I, there was no immunonephelometric change (Figures 2 and 3), whereas detergent II and III still caused a decrease in the rate of light-scattering development. We therefore interpret the data to mean that alkylaminepolyoxyethylene specifically binds to phospholipids located at the surface of VLDL. This interaction seems to be favored by electrostatic forces between the amine group of the detergent and negative polar groups of phospholipid and by hydrophobic forces between the alkyl chain of the detergent and acyl groups of phospholipids. The
use of detergent I instead of detergent III (2) permits the
omission of enzymic pretreatment of hyperlipidemic
serum.

The correlation between results of apolipoprotein B de-
termination by radial immunodiffusion and nephelometry in
which detergent I is used was similar to that after the recently
described procedure (2), as estimated from 255 serum sam-
pless: \( y_{NID} = 0.79x + 0.200 \) (correlation coefficient, 0.91). Of
these, 76 serum samples were from normolipemic subjects, and
the others were from subjects with various forms of hyperli-
poproteinemia. Triglyceride concentrations in the samples
ranged from 0.49 to 29.5 g/L, cholesterol ranged from 150 to
9450 mg/L. Twenty-nine serum samples were ultracentrifuged
before the analysis for apolipoprotein B in whole serum and
in the subnatant fraction. The data correlated satisfactorily,
with almost identical lines of regression for the two series
(Figure 4). In our experience the simplified procedure seems
to be more suitable than the preincubation method for clinical
and screening purposes because the cost for an assay is con-
siderably less and handling or storing of enzymes is avoided.
This procedure can also be applied to nephelometric deter-
mination of other apolipoproteins.

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Evaluation of Automated Enzyme Immunoassays for Five
Anticonvulsants and Theophylline Adapted to a Centrifugal Analyzer

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We report a clinical evaluation of the enzyme immu-
noassay (EMIT) performed with the GEMSAEC centrifugal
analyzer as compared to gas–liquid and liquid chromato-
graphy for anticonvulsant drugs and theophylline, re-
spectively. A good correlation was obtained for all drugs,
although some difficulties were experienced with one lot of
reagent for ethosuximide. The analyzer has an economic
advantage if many samples are being analyzed for few
drugs in each sample.

Additional Keyphrases: monitoring therapy – economics of laboratory operation – gas- and liquid-chromatography compared

The “Enzyme Multiplied Immunoassay Technique”
(EMIT; Syva Corp., Palo Alto, CA 94304) is widely used for
monitoring therapeutic drugs in serum. Several communica-
tions have dealt with adaptation of the EMIT reagents to
various centrifugal analyzers for the determination of pheno-
ytoin, phenobarbital, and theophylline (1–7), and precision
has been reported to be in the 4–15% range, with acceptable
correlations with other methods.

We report here a clinical evaluation in which we used EMIT
with the GEMSAEC centrifugal analyzer (Electro-Nucleonics,
Inc., Fairfield, NJ 07006) as proposed by Syva Corp. for phen-
obarbital, phenytoin, primidone, carbamazepine, ethosux-
ime, and theophylline. Results for unselected routine patient
specimens were compared with those obtained by column-
chromatographic methods.

Materials and Methods

Reagents

EMIT reagents used with the GEMSAEC (phenytoin
6B019-G02, phenobarbital 6D019-G02B, primidone
6C119-G02B, carbamazepine 6F119-G04A, ethosuximide
6E119-G03, and theophylline 6P019-G03C) were kindly
supplied by Syva Corp. An EMIT ethosuximide kit, Reagent
A 6E118-H01 and Reagent B 6E148-H01, containing an
improved antibody was also tested. The commercially available
antiepileptic drug calibrators from Syva were used to calibrate
the GEMSAEC and the gas–liquid chromatograph. Theo-
phylline calibrators from Syva were used to calibrate the
GEMSAEC. A standard containing 20 mg of theophylline per

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