The VLDL revealed an apo E4/3 heterozygous pattern. We found that the plasma was thus found to be apo E4/3 heterozygous. An additional component of the band is thus clearly identified as SAA2 by specific antibodies to SAA. In some other cases, apo E4 was reported when it actually was not present, e.g., in VLDLs from plasma in lane A of Figure 1.

Recently Stuyt et al. (6) reported on the co-focusing with apo E4 of additional proteins in VLDL from patients with acute pancreatitis. Our results demonstrate that one of these co-focusing proteins can be serum amyloid A (isoform SAA2), which helped to explain the different reports (7, 8) on apo E4 frequencies in type V hyperlipoproteinemia. We show here that immunological identification of apolipoproteins avoids the possible confusion of SAA2 with apo E4.

We (9) and others (10), by setting up immunoblotting procedures for phenotyping of apo E, have seen the possible confusion of the apo E pattern with serum amyloid A when apo VLDL protein staining is used to determine phenotypes instead of immunological detection. One should therefore be aware of this phenomenon when interpreting apo E phenotyping results and should always run an apo E4 standard when immunoblotting is not available.

Fig. 1. Immunoblot of serum with antihuman apolipoprotein E and antihuman serum amyloid A (SAA) antibodies

The serum was isofocused at a pH range from 4 to 7; the proteins were then transferred to nitrocellulose and developed with antibodies specific for apo E (A, B) or SAA (C). Lane A shows an apo E3/2, lane B an apo E4/3 heterozygous pattern. Note that the SAA2 isoform almost co-focuses with apo E4. Lane B and C contain the same serum from a patient who was initially phenotyped by apo VLDL isofocusing and subsequent Coomassie Blue staining as apo E4/4 heterozygous. Immunoblot revealed that the prominent band seen before in the apo E4 position upon apo VLDL focusing consisted of apo E4 and SAA. The pattern revealed by immunoblotting was found to be apo E4/3. Serum A also exhibited a band in the apo E4 region upon VLDL isofocusing. Immunoblotting revealed the band as SAA.

Apolipoprotein (apo) E is a polymorphic apolipoprotein with mainly three isoforms in plasma—E2, E3, and E4—that differ in cysteine and arginine content (4) and are coded for by three alleles e2, e3, and e4. Six phenotypes can be distinguished—three homozygotes (E2/2, E3/3, and E4/4) and three heterozygotes (E2/3, E2/4, and E3/4)—on isofocusing, mainly performed on urea-soluble apolipoproteins from VLDLs (5).

We could demonstrate that coincident with a massive increase in serum amyloid A, the acute-phase proteins associate with VLDLs and are co-isolated with VLDLs by ultracentrifugation. They are urea-soluble apolipoproteins and are separated by isoelectric focusing together with the usual VLDL apolipoproteins, SAA2 almost co-focusing with apo E4 with which it can thus be confused, leading to false phenotyping of apo E when protein staining is used to interpret the phenotypes. After Coomassie Blue staining, the SAA-containing VLDL may be looked at as an apo E4-containing pattern. A more reliable procedure for discriminating the proteins focusing at the apo E4 position would seem to be the transfer of apolipoproteins, after isofocusing, to nitrocellulose sheets and their identification by use of specific antibodies to apo E. As shown in Figure 1, apo E4 and SAA2 almost co-focus. In the Figure they are discriminated by specific antibodies both to apo E and SAA. Initially the VLDL phenotyped for apo E from lane B was seen with a pronounced band in the apo E4 position, interpreted as an apo E4 homoygous pattern. Isofocusing of plasma and subsequent immunoblotting revealed that the band found in the apo E4 position consisted both of apo E4 and SAA (Figure 1, B, C). The plasma was thus found to be apo E3/4 heterozygous. An additional component of the band is thus clearly identified as SAA2 by specific antibodies to SAA. In some other cases, apo E4 was reported when it actually was not present, e.g., in VLDLs from plasma in lane A of Figure 1.

References
wich-immunoassay methodology (1); the solid phase consists of glass fiber papers. We evaluated the performances of ferritin determination in serum on this system according to the general protocol proposed by the French Committee for Validation of Methods (2).

Reproducibility was tested in two laboratories with Tri-Rac controls (Baxter-Travenol) (n = 20); for concentrations of 66 and 163 µg/L, the within-run CVs were 1.9% and 3.2%, respectively. Between-day (n = 30) CVs were <6% for concentrations of 66, 152, and 260 µg/L.

Accuracy was checked by comparison of the Stratus calibrators with the international standard recommended by WHO (ref. 80/602). Bias between expected and experimental values range from −3.3% to 6.1%, depending on the dilutions. The between-sample carryover is constant (0.05%) and can be neglected for ferritin concentrations up to 5000 µg/L. The Stratus system exhibited no high-dose hook-effect up to 245 000 µg/L—far higher than the upper limits given by other commercial kits. Immunological reaction is linear up to 750 µg/L.

The detection limit was assessed by geometric dilutions of a hypoferritinemic serum in zero calibrator; it was also calculated from zero calibrator values of each run. The calculated detection limit was 2.6 µg/L.

Stratus results (y) were compared with those of EIA-Ferrizyme (x) (Abbott Diagnostic, Chicago, IL) and of IRMA-Magic (x') (Corning Medical, Medfield, MA). Results exceed those of the EIA-Ferrizyme but correlate well. The linear regression equations are

\[ y = 1.43x + 1.3 \text{ µg/L for 55 subjects with iron deficiency,} \]

\[ y = 117x + 1 \text{ µg/L for 40 healthy subjects,} \]

\[ y = 1.3x - 123 \text{ µg/L for subjects with hyperferritinemia (iron overload, inflammatory diseases, hematologic malignancies, and solid tumors).} \]

In contrast, the Stratus results are lower than those of the IRMA method; the linear regression equation is

\[ y = 0.87x + 0.6 \text{ µg/L for 78 subjects.} \]

In all cases we never observed any discrepancy between sample results and the clinical groups to which the specimens belonged. Our observations emphasize the necessity of referring to appropriate reference values.

We conclude that precision, accuracy, quickness (8 min for a result if calibration curve is stored), and practicability are the main features of the Stratus System.

References

Glyoxal: An Artefact from Metronidazole and Glyoxylic Acid In Urinary Organic Acid Analysis, G. A. Mills; V. Walker; and D. L. Corina* (1 Clinical Biochemistry, University of Southampton, Southampton General Hospital, Southampton, S09 4XY, U.K.; and 2 Dept. of Biochemistry, University of Southampton, Southampton, S09 5NH, U.K.)

During analysis for organic acids in urine samples from babies and children, with a procedure that incorporates an oximation step with hydroxylamine hydrochloride at alkaline pH to stabilize oxo-groups (1, 2), we sometimes observed two large peaks in capillary gas chromatograms, with methylene units 11.81 and 11.85 (on OV-101 stationary phase). Electron-impact mass spectrometry showed the unknown compounds to be isomeric (Figure 1). Their apparent relative molecular mass was 232, as confirmed by chemical ionization with isobutane as reactant gas, and the ion fragment at m/z = 147 suggested that the molecule contained two trimethylsilyl (TMS) groups. The peaks disappeared when the oximation step was omitted from the analysis, indicating the presence of either oxo or aldehyde functional groups. The even molecular mass suggested the compound contained two oxime derivatives. Of possible substances fulfilling these criteria, glyoxal [HCOCHO] seemed likely. Chromatographic and spectroscopic analysis of the authentic compound corroborated this hypothesis. This compound has not been reported in urine before.

High concentrations of glyoxal were observed in samples from patients receiving the antimicrobial drug metronidazole [Flagyl]; 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole]. This drug, which is in part excreted unchanged in urine after administration (3), has a 2-hydroxyethyl sidechain, which could be converted to glyoxal after cleavage from the imidazole ring during analysis. Metronidazole subjected to our oximation procedure was found to produce glyoxal, indicating that it probably was the source of the compound in these patients.

In pursuing this investigation, we observed that glyoxal was also produced during oximation of the normal endogenous urinary constituent, glyoxylic acid [HCOOCHO]. This probably accounted for the small peaks of glyoxal that we commonly saw in chromatographic profiles of normal urine samples.

Awareness of this previously unreported artefact is important to laboratories where a similar analytical procedure is used for urinary organic analysis.

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References