An assay of serum pancreatic amylase was adapted to various analyzer systems by modifying a kit for total amylase determination. The salivary amylase fraction of total amylase was inhibited by an inhibitor protein from wheat germ (cat. no. A-3535; Sigma Chemical Co., St. Louis, MO) (1, 2). I mixed 25 μL of inhibitor solution (1.6 μg/mL in 10 g/L human serum albumin solution) with 25 μL of serum sample and precultivated for 5 min at 25 °C. Then 250 μL of a solution of twofold-diluted (with water) reaction mixture "Testomar Amylase", Behring, Marburg, F.R.G.) and additional inhibitor (1.6 μg/mL final concentration) was added. After a 15-min lag phase (at 25 °C), reaction-rate curves were measured photometrically in fast-rotating cuvette rings (Megalyzer System MFT; Medizinische Feinwerktech-nik, Marburg, F.R.G.). A computer program calculated units of pancreatic amylase activity. Within 30 min, 120 analyses could be performed.

Efficiency of the inhibiting procedure was tested with pancreatic and salivary extracts. The inhibitor from wheat germ inhibited 40% of the activity of pancreatic amylase and 96% of the salivary amylase.

The reference interval for serum pancreatic amylase determined on 200 apparently healthy persons was 4 to 37 U/L. (In the 40% inhibition of pancreatic amylase, I corrected for the calculation of activity; the remaining 4% of salivary amylase activity was neglected.) The assay's standard curve was linear to 200 U/L. For comparison studies, I also determined pancreatic amylase activities of 50 sera with a nonkinetic isoeamylase test (Phadebas; Pharmacia, Uppsala, Sweden). Results by both methods correlated well (r = 0.94).

Previous inhibitor methods described for pancreatic amylase analysis have suffered from nonlinear reaction-rate curves. Deviations from linearity may result from dissociation of the enzyme inhibitor complex by substrate interaction (3). The reaction-rate curve I obtained is strictly linear. Presumably the presence of excess inhibitor in the enzyme reaction assay results in an equilibrium (after lag phase) between dissociation and binding of inhibitor and substrate to the enzyme and the reaction-rate curve is a precondition for kinetic measurements in many analyzers. Combined with inhibitor specificity and low costs per assay, this method allows routine determination of pancreatic amylase.

References

Serum Fructosamine and Thyroid Function, D. Lloyd and J. Marples (Dept. of Clin. Biochem., Royal Albert Edward Infirmary, Wigan Lane, Wigan WN1 2NN, U.K.)

For assay of serum fructosamine to be reliable as an index of glyceria over the preceding one to three weeks, it is assumed that both concentration and turnover rate of serum proteins do not differ sufficiently from those in the reference population to affect the degree of protein glycation.

However, this is not the case when serum albumin concentrations are <30 g/L (1, 2). Here, we report that patients with abnormal rates of serum protein turnover also have fructosamine concentrations that differ significantly from those of the reference population.

Thyrototoxicosis was chosen as a model for increased serum protein turnover, hypothyroidism for decreased turnover. We measured fructosamine, total protein, and glucose (samples centrifuged within 30 min of collection) in serum of 50 untreated non-diabetic hypothyroid patients (free thyroxin index <15 nmol/L and thyrotropin >15 milli-int. units/L, normal 0–9).

The reference population consisted of 100 non-diabetic euthyroid outpatients.

<table>
<thead>
<tr>
<th>Fructosamine</th>
<th>Total protein</th>
<th>Glucose (random)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (and SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thyrotoxic</td>
<td>1.95 (0.22)</td>
<td>66.7 (5.1)</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>2.53 (0.40)</td>
<td>70.7 (6.1)</td>
</tr>
<tr>
<td>Controls</td>
<td>2.30 (0.23)</td>
<td>68.0 (4.7)</td>
</tr>
</tbody>
</table>

Statistical difference from control population: * p <0.001, ** p <0.05, * not significant.

The results tabulated above show a significant decrease in serum fructosamine concentration in the thyrotoxic patients and a less significant increase in the hypothyroid patients as compared with the euthyroid controls. These differences cannot be accounted for by changes in protein or glucose concentrations in serum, but presumably must result from increased and decreased protein metabolism, respectively. We conclude that fructosamine results for patients in whom there is likely to be abnormal serum protein turnover must be cautiously interpreted.

References

Free 5-Hydroxytryptamine in Plasma: Fact or Artifact?, F. Artigas, J. Ortiz, M. J. Barrias, E. Martinez, and E. Gelpi (Dept. of Neurochemisty, CSIC, J. Girona Salgado, 18-26 Barcelona, Spain)

Because serotonin (5-hydroxytryptamine, 5HT) is highly concentrated in platelets, its concentration in plasma may be overestimated owing to an artificial platelet contribution. This has produced some confusion in the literature. The scarce data on plasma 5HT have been obtained with different sampling and analytical conditions and thus reported concentrations range from undetectable to almost micromolar. Previous results from this laboratory indicate the existence of a non-particulate plasma pool of 5HT, which under different pharmacological treatments [inhibition of 5HT synthesis (1) and inhibition of 5HT uptake by platelets (submitted)] behaves independently of platelet 5HT. A clear-cut difference between both 5HT pools is thus suggested. However, better to characterize the serotoninergic status of blood samples, we have analyzed 5HT in platelets and in platelet-free plasma (PFP) from 58 healthy people; ages 36 ± 12 yr; body weight 64 ± 12 kg; 28 men, 30 women;
platelet count: 271 000 ± 53 000 platelets/mm³ of blood. The sampling and analytical procedures were as described elsewhere (7). Briefly, blood was collected (8:30–10:00 a.m.) into K₃ EDTA-containing tubes. After taking aliquots for platelet counting and liquid chromatographic–fluorimetric analysis of 5HT (2) we centrifuged the blood twice (15 and 30 min, at 1000 × g) to obtain PFP. The 5HT in plasma was extracted with n-butanol:heptane, with bufotenine (N,N-dimethyl serotonin) as the internal standard. Extracts were analyzed by liquid chromatography with amperometric (+0.55 V) detection. We obtained the following results (x ± SD): plasma 5HT 5.0 ± 3.5 nmol/L, platelet 5HT 3.89 ± 1.7 nmol/10⁶ platelets.

Thus, 5HT in the low nanomolar range is consistently detectable in plasma. Moreover, correlation of plasma 5HT concentrations vs platelet 5HT was not significant (r = 0.191). All this evidence, taken together, suggests that under our working conditions plasma 5HT is not an artifact originating from 5HT in platelets but is a clearly different biochemical entity.

References

Stability of Subfractions of High-Density Lipoproteins in Stored Sera, Klaus Jung, Wolfram Blank, and Dietmar Schols (Dept. of Exptl. Organ Transplantation, University Hospital Charité, Humboldt University Berlin, Lenineallee 49, DDR-1017 Berlin, G.D.R.)

Differential determination of subfractions of high-density lipoproteins (HDL) is increasingly important, because HDL₂ seems to be a better risk indicator for atherosclerosis than is total HDL cholesterol. Conventional ultracentrifugation methods for determining HDL subfractions are time consuming and impractical for large-scale screening, so interest focuses on methods based solely on precipitation (1–3). Often it is desirable to perform these measurements on stored samples. Results concerning the stability of HDL cholesterol in stored sera are contradictory (4); exact data on the stability of HDL subclasses in such samples are not yet available. Therefore, we have examined HDL subfractions in stored sera of healthy persons and of renal-transplant recipients, using a precipitation method. Because the precipitation method of Gidez et al. (1) has several drawbacks and the test based on precipitation with polyethylene glycol (PEG) 20 000 of different concentrations (3) is only commercially available, we decided to apply the method according to Lundberg et al. (2), in which PEG 6000 is used to precipitate all lipoproteins except HDL in the first step and dextran sulfate to precipitate HDL₂ in the second step.

For 10 healthy persons [mean cholesterol 1.607 (SD 0.212) g/L, mean triglycerides 1.166 (SD 0.389) g/L] and 10 renal-transplant recipients [mean cholesterol 2.587 (SD 0.395) g/L, mean triglycerides 1.650 (SD 0.483) g/L], total HDL cholesterol amounted to 584 mg/L (SD 126) and 659 mg/L (SD 155), respectively. During four-day storage of the sera at 4 °C and −20 °C the total HDL cholesterol concentration did not change significantly (p < 0.05). However, storage has a different effect on the HDL subclasses in both groups.

Storage, days °C Healthy persons Renal-transplant recipients

<table>
<thead>
<tr>
<th>HDL₄ cholesterol concn, mean ± SD, mg/dL*</th>
<th>40.6 ± 7.7 (+10.9)b</th>
<th>40.8 ± 9.9 (+35.1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>36.5 ± 3.6</td>
<td>30.2 ± 4.7</td>
</tr>
<tr>
<td>1/4 °C</td>
<td>37.2 ± 3.8 (+1.6)</td>
<td>30.8 ± 5.7 (+1.9)</td>
</tr>
<tr>
<td>2/4 °C</td>
<td>37.1 ± 3.8 (+1.4)</td>
<td>32.8 ± 6.9 (+8.6)b</td>
</tr>
<tr>
<td>3/4 °C</td>
<td>37.3 ± 3.9 (+1.9)</td>
<td>36.8 ± 7.3 (+21.8)</td>
</tr>
<tr>
<td>4/4 °C</td>
<td>38.3 ± 3.9 (+4.6)</td>
<td>39.6 ± 7.9 (+31.1)</td>
</tr>
</tbody>
</table>

* n = 10. Nons, in parentheses indicate percentage change from values obtained on day of sample collection.

b Significantly different by paired t-test, p < 0.05.

In renal-transplant recipients, HDL₄ cholesterol increased considerably during storage at 4 °C or −20 °C. Already on the second day an increase of about 10% in HDL₄ cholesterol could be observed, whereas for healthy persons HDL₄ cholesterol increased only in frozen samples. Evidently, the HDL₄/HDL₃ interconversion differs between healthy persons with normal composition of lipoproteins and patients such as renal-transplant recipients showing abnormal lipoprotein composition. Thus we recommend determination of HDL subclasses by the precipitation procedure according to Lundberg et al. (2) not later than 24 h after blood is sampled. Kostner et al. (3), using the precipitation with different PEG concentrations, described identical values for HDL subclasses after storage of sera at 4 °C for up to one week. Therefore, this method seems to be more favorable in this respect. However, they did not mention whether they investigated samples from healthy persons or from patients.

References

Effect of Storage Temperature on the Precipitation of Albumin from Urine, J. C. Townsend (Dept. of Clin. Chem., Royal Hallamshire Hospital, Sheffield S10 2JF, U.K.)

I collected timed urine specimens from 15 healthy volunteers to investigate methods of avoiding the precipitation of albumin in frozen-stored urine samples (1). Aliquots of each urine collection were stored at room temperature (with sodium azide as preservative), at 4 °C and −20 °C. Each urine collection was centrifuged and the supernate was also stored at −20 °C.