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 preincubated for 5 min at 25 °C. Then 250 μL of a solution of twofold-diluted (with water) reaction mixture "Testostar 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 (Megalizer System MFT; Medizinnische Feinwerktechnik, 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 isoamylase 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. In the reference method, this 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 glycemia 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.

Thyrotoxicosis 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 thyroidropin >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, *p 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?, P. Artigas, J. Ortiz, M. J. Sarrias, E. Martinez, and E. Gelpl (Dept. of Neurochemistry, 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 serotonergic status of blood samples, we have analyzed 5HT in platelets and in platelet-free plasma (PPP) from 58 healthy people; ages 36 ± 12 yr; body weight 64 ± 12 kg; 28 men, 30 women;