Optimized serum pancreolauryl test for differentiating patients with and without chronic pancreatitis

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The serum pancreolauryl test has limited sensitivity for detecting mild pancreatic insufficiency. The aim of this study was to optimize the serum pancreolauryl test so as to increase the probability of positive results in patients with chronic pancreatitis. The study had three parts. First, the sampling time was optimized by analyzing retrospectively the frequency of fluorescein peaks at different times from 0 to 240 min in 560 consecutive patients. Second, the calculation of serum fluorescein concentrations by means of a standard calibration factor was prospectively compared in 271 consecutive patients with a modification involving a specimen-specific calibration factor for each patient. Third, the clinical utility of the intravenous injection of secretin before ingestion of the test meal was prospectively evaluated in a further 32 patients. As a result, the optimized serum pancreolauryl test developed differs from the former version of the test in utilizing intravenous administration of secretin before the test meal, calculation of serum fluorescein based on specimen-specific calibration factors, and blood samples taken only at 0 (basal), 120, 150, 180, and 240 min. This optimized pancreolauryl test was abnormal more frequently in patients with chronic pancreatitis than was the formerly used test, especially for cases of mild and moderate disease.

Diagnosis of chronic pancreatitis is based on the demonstration of morphological and functional changes that appear within the pancreas as a consequence of the disease (1). Staging of chronic pancreatitis is based on the degree of these morphological and functional changes (1). Morphological changes within the pancreas can be demonstrated by ultrasonography (US),¹ computed tomography (CT), and endoscopic retrograde pancreatography (ERP), ERP being considered the most accurate procedure for both diagnosing and staging chronic pancreatitis (1). Because ERP is invasive and is associated with complications, however, repeating this procedure for the follow-up of chronic pancreatitis is discouraged. Also, CT cannot be routinely used for following up patients with uncomplicated chronic pancreatitis because of the use of radiation, and US, although noninvasive, is not sensitive enough (1).

Among tests to evaluate pancreatic function, the secretin–cholecystokinin or secretin–caerulein test is the most accurate. This test is, however, invasive, expensive, not generally available, and, therefore, inapplicable for the clinical routine. Tubeless pancreatic function tests, in contrast, are easily applicable to clinical practice and are useful not only for supporting the diagnosis of chronic pancreatitis but also for monitoring patients with this disease (1). Among tubeless pancreatic function tests, the serum pancreolauryl test (PLT), as modified by our group (2), is used to evaluate exocrine pancreatic function and to grade the functional impairment of the gland (3). In the PLT, fluorescein dilaurate is administered orally together with a standardized breakfast. A pancreas-specific cholesterol ester hydrolase acts on this compound, releasing water-soluble fluorescein, which is absorbed from the gut. Administration of secretin just before and metoclopramide immediately after ingestion of the breakfast promotes a more rapid peak for fluorescein concentration in serum and seems to enhance the discrimination between unaffected subjects and patients with moderate impairment of pancreatic function (2). This modified PLT has a sensitivity of 82% and specificity of 91% for the detection of exocrine pancreatic dysfunction in chronic pancreatitis.

¹ Nonstandard abbreviations: US, ultrasonography; CT, computed tomography; ERP, endoscopic retrograde pancreatography; PLT, pancreolauryl test; and CI, confidence interval.
its results correlate significantly with the bicarbonate output after secretin stimulation (2)—considered to be the most sensitive individual secretory marker in patients with chronic pancreatitis (1)—and with the degree of morphological changes detected by ERP (4). The sensitivity of the PLT for the detection of pancreatic dysfunction in patients with mild morphological changes of chronic pancreatitis, however, is far from optimal, at best 52% (3).

Our aim in the present series of studies was to further optimize the PLT, to increase the probability of obtaining positive test results in patients with chronic pancreatitis. With this purpose, we optimized the blood sampling times and the calculation of serum fluorescein concentration. Furthermore, because the cost of the test increases markedly if secretin is administered, we reevaluated the utility of this previously described modification (2).

**Materials and Methods**

**Optimization of Sampling Time**
In this retrospective cohort study, all patients referred to our gastrointestinal laboratory for PLT during the last 3 years were studied. The inclusion criterion therefore was the clinical indication for evaluation of exocrine pancreatic function. Potential spectrum bias was avoided by including all consecutive patients (no exclusion criterion was considered). In all, 560 patients (418 men, 142 women, mean age 48 years, range 16–89 years) were studied. In all cases, fluorescein was measured in blood samples taken before (basal) and at 30, 60, 120, 150, 180, and 240 min after ingestion of a standardized breakfast. The breakfast, consisting of 40 g of wheat bread, 20 g of butter, and 200 mL of tea, together with two 0.5-mmol capsules of fluorescein dilaurate (blue capsules, Pancreolauryl-Test-N; Temmler Pharma), was given after an overnight fast and immediately after intravenous administration of secretin, 1 U/kg body weight. To facilitate simultaneous gastric emptying of the fluorescein and the meal, the capsules were broken open and the total contents spread on the bread together with the butter. Metoclopramide (10 mg) was injected intravenously after the subjects ingested the breakfast.

Fluorescein concentration was measured in duplicate in all serum samples as follows: 500 μL of serum was mixed with 500 μL of 0.5 mol/L ethanolic KOH and incubated for 60 min at 70 °C. After cooling, 1 mL of 0.15 mol/L magnesium sulfate solution was added, mixed, and centrifuged at 2671 g for 10 min. The absorbance of the supernatant was measured photometrically at 492 nm against that of the basal serum sample, and the mean for each serum sample was multiplied by 20.83 (factor previously calculated in our laboratory from a calibration curve defined by progressive dilution of fluorescein disodium in a serum pool (2)) to convert absorbance into concentration units (mg/L). The maximal serum fluorescein concentration (peak) is considered as the result of the test. The PLT result indicates normal pancreatic function if the peak is >4.5 mg/L (2, 3). The frequency of fluorescein peaks at each sampling time was calculated.

**Optimization of Calculations of Serum Fluorescein Concentration**
The last 271 consecutive patients (191 men, 80 women, mean age 49 years, range 19–89 years) of the 560 patients described above were prospectively included in this part of the study. Of these, 127 patients had chronic pancreatitis; 24 mild, 48 moderate, and 55 severe, based on the findings by US, CT, and ERP according to previously reported criteria (5–7). Diagnosis of chronic pancreatitis relied on the demonstration of specific morphological changes in the pancreas as evidenced by ERP, CT, and US (5–7). Imaging findings (US, CT, and ERP) were evaluated by investigators unaware of the results of pancreas function tests. The remaining 144 patients had other pancreatic or gastrointestinal extrapancreatic disorders; chronic pancreatitis had been initially suspected because of abdominal pain and (or) suspected maldigestion. The final diagnoses in these patients were liver disease (11), common bile duct stones (10), inflammatory bowel disease (12), gastrointestinal tumors including hormone-producing tumors (8), malabsorption due to sprue or infectious gastroenteritis (13), maldigestion associated with partial gastrectomy (10), and functional disorders [functional dyspepsia and (or) irritable bowel syndrome; 34]; 28 more patients were in the recovery phase of acute pancreatitis, and 18 had pancreatic cancer.

A specimen-specific calibration factor for fluorescein was calculated as follows: 44 mg of the content of the red Pancreolauryl-Test-N capsules, corresponding to 10 mg of fluorescein disodium, was diluted in 100 mL of distilled water. This solution is stable for at least 1 week at 4 °C (preliminary study). This fluorescein disodium solution (60 μL) was added to 1440 μL of basal serum sample from the patient (final fluorescein concentration of this control sample, 4 mg/L) and processed the same as the remaining serum samples for fluorescein determination. The specimen-specific factor (F) was calculated as follows: $F = 4/(A_{C0} - A_0)$, were $A_{C0}$ and $A_0$ are the absorbances of the control sample and the basal sample, respectively. The result of the test was calculated by multiplying the mean absorbance of each serum sample collected during the test (at 30, 60, 120, 150, 180, and 240 min) by both the standard calibration factor [20.83 (2)] and the specimen-specific calibration factor.

For interpretation of results, we assumed that an abnormal PLT result defines the presence of pancreatic dysfunction, a condition commonly seen in chronic pancreatitis. Thus, although no “gold standard” was used to define pancreatic dysfunction, we assessed the accuracy of PLT, as calculated with both calibration factors, and expressed the sensitivity [(positive tests in chronic pancreatitis/total with chronic pancreatitis)×100] and specificity [(negative tests in patients without chronic pancreatitis/total without chronic pancreatitis)×100] and the
accompanying 95% confidence intervals (CI95) of the calculations. Though testing of referral patients seen at a university hospital may be a cause of selection bias, inclusion of all consecutive patients in whom a pancreatic function test was indicated makes the prevalence of chronic pancreatitis in this collective potentially more representative of a more general setting. With this assumption, we also calculated the positive and negative predictive values [(total with chronic pancreatitis/total positive tests)×100 and (total without chronic pancreatitis/total negative tests)×100, respectively] and their CI95.

A test was considered positive if the serum fluorescein peak (highest fluorescein concentration measured during the test) was <4.5 mg/L (2, 3). As previously mentioned, chronic pancreatitis was defined by the presence of specific morphological changes in the ERP, CT, and US in patients with symptoms suggestive of the disease (abdominal pain, chronic diarrhea, or both) and in whom other differential diagnoses were adequately excluded. Differences in accuracy were tested by applying a binomial test for comparison of percentages. We also constructed ROC curves for these values and calculated the areas under the ROC curves.

**VALIDATION OF UTILITY OF INTRAVENOUS ADMINISTRATION OF SECRETIN**

In this prospective controlled cross-over study, which included another 32 ambulatory patients (16 men, 16 women, mean age 50 years, range 36–82 years), PLT was performed as described above (with a specimen-specific calibration factor and collection times at 0, 30, 60, 120, 150, 180, and 240 min) and was repeated 2 days later in the same way but without administration of secretin. Among these patients, 16 were diagnosed as having chronic alcoholic pancreatitis; the remaining 16 had other extrapancreatic gastrointestinal disorders (8 with functional gastrointestinal disorders, 3 with chronic inflammatory bowel disease, 3 with malabsorption due to sprue, and 1 each with liver cirrhosis and a secretory diarrhea). Patients with chronic pancreatitis were divided into mild (n = 4), moderate (n = 7), and severe (n = 5) cases. Diagnosis and staging of chronic pancreatitis were performed by following the same criteria described above. The data collected were compared by means of the paired nonparametric Wilcoxen test: maximal serum fluorescein concentration (peak), time to peak, and area under the serum fluorescein concentration curve throughout the test (240 min).

All procedures followed in the present studies were in accordance with the Helsinki Declaration of 1975, as revised in 1983. Guidelines for studies of clinical performance of laboratory tests were followed.

**Results**

Because the result of the PLT is defined by the serum fluorescein concentration peak, optimization of the sampling time was based on an analysis of the frequency of fluorescein peaks at different sampling times from 0 to 240 min after ingestion of the test meal. Optimization of the calculation of serum fluorescein concentration was based on the comparison between the results obtained with the application of a standard calibration factor to all patients (as usually done) and those obtained with the application of a specimen-specific calibration factor (an individual factor for each patient).

**OPTIMIZATION OF THE SAMPLING TIME**

The peak serum fluorescein value was never reached in any patient by 30 or 60 min after the test meal. The peak was reached at 120 min in 140 patients (25%), at 150 min in 162 patients (29%), and at 180 min in 196 patients (35%). In the remaining 62 patients (11%), the greatest recorded value of fluorescein was reached at 240 min; however, the possibility of a further increase of the fluorescein concentration cannot be excluded in these cases because no more fluorescein determinations were made after this time.

**OPTIMIZATION OF THE CALCULATION OF SERUM FLUORESCEIN CONCENTRATION**

The mean ± SE individual calibration factor obtained in the 271 patients studied was 17.4 ± 0.14. The sensitivity of the PLT for chronic pancreatitis (rate of positive results in this disease condition) obtained by applying both the previous standard calibration factor and the specimen-specific calibration factor is shown in Table 1. For a cutoff of 4.5 mg/L in both methods, the sensitivity of the PLT for mild and moderate chronic pancreatitis increased by 30% when applying the specimen-specific calibration factor (Table 1). The specificity (rate of negative results in patients without chronic pancreatitis) and positive predictive value of the test were independent of the calibration factor.

**Table 1. Accuracy of the PLT for chronic pancreatitis (CP) according to the calibration factor applied for the calculation of the serum fluorescein concentration.**

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Standard factor (20.83)</th>
<th>Specimen-specific factor</th>
<th>Binomial test</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sensitivity</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Total with CP</td>
<td>127</td>
<td>79 (72–86)</td>
<td>95 (90–98)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Mild CP</td>
<td>24</td>
<td>46 (28–65)</td>
<td>75 (55–88)</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>Moderate CP</td>
<td>48</td>
<td>71 (57–82)</td>
<td>100 (93–100)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Severe CP</td>
<td>55</td>
<td>100 (93–100)</td>
<td>100 (93–100)</td>
<td>NS*</td>
<td></td>
</tr>
<tr>
<td><strong>Specificity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total without CP</td>
<td>144</td>
<td>85 (79–91)</td>
<td>81 (75–88)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Extrapancreatic</td>
<td>98</td>
<td>94 (87–97)</td>
<td>91 (83–95)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Pancreatic diseases other than CP</td>
<td>46</td>
<td>65 (51–77)</td>
<td>61 (46–74)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td><strong>Predictive value</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Positive</td>
<td>82</td>
<td>82 (76–88)</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>82</td>
<td>82 (75–89)</td>
<td>NS</td>
<td>0.001</td>
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</table>

* NS, not significant.
factor used, whereas the negative predictive value increased significantly by applying the specimen-specific calibration factor (Table 1). In a threshold-independent evaluation (Fig. 1), the accuracy of the specimen-specific calibration factor-based PLT was also greater than that of the standard calibration factor-based PLT. The ROC curves of both tests were very similar at thresholds <3.0 mg/L and >5.5 mg/L (zone for sensitivity <0.5 and specificity <0.6, respectively). At the threshold range of greatest clinical relevance (PLT result between 3.0 and 5.5 mg/L), however, the accuracy of the test applying the specimen-specific calibration factor was clearly greater than that of the test based on the standard calibration factor. Thus, when we limited the ROC graphic to the zone of sensitivity >0.5 and specificity >0.6 (maximal area under the ROC curve = 0.20; Fig. 1), the area under the ROC curve was 0.15 when the specimen-specific calibration factor was applied and 0.09 when the standard calibration factor was used (P < 0.01).

VALIDATION OF THE UTILITY OF INTRAVENOUS ADMINISTRATION OF SECRETIN
PLT curves obtained with and without intravenous administration of secretin before the test meal are shown in Fig. 2. Administration of secretin markedly influenced the test result in the patients with chronic pancreatitis but not in those with extrapancreatic diseases. The result of the test (peak) and the area under the serum fluorescein concentration curve decreased significantly (P < 0.001) when secretin was given in patients with chronic pancreatitis but not in those with extrapancreatic diseases (Table 2). All patients with chronic pancreatitis had a pathological PLT result when secretin was administered; however, all 4 patients with mild chronic pancreatitis, 2 of 7 patients with moderate chronic pancreatitis, and 1 of 5 patients with severe chronic pancreatitis had a normal PLT result when secretin was not administered (Fig. 3). The sensitivity of the PLT for chronic pancreatitis was therefore 100% (CI95 81–100%) when secretin was given and 56% (CI95 33–77%) when secretin was not given (P < 0.01). The specificity of the PLT did not change with the intravenous administration of secretin, and all 16 patients studied with extrapancreatic diseases had a normal PLT result, whether there was a previous secretin infusion or not (specificity 100%, CI95 81–100%). The time to peak was not influenced by secretin infusion in any of the patients (Table 2).
Table 2. Effect of secretin administration before ingestion of the test meal on serum fluorescein concentration peak, area under the serum fluorescein concentrations throughout the test (AUC), and time to peak in patients with chronic pancreatitis (n = 16) and gastrointestinal extrapancreatic (GI) disorders (n = 16).

<table>
<thead>
<tr>
<th></th>
<th>Without secretin</th>
<th>With secretin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic pancreatitis</td>
<td></td>
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</tr>
<tr>
<td>Peak, mg/L</td>
<td>4.3 ± 0.4</td>
<td>2.8 ± 0.3a</td>
</tr>
<tr>
<td>AUC, min · mg/L</td>
<td>585 ± 72</td>
<td>392 ± 44a</td>
</tr>
<tr>
<td>Time to peak, min</td>
<td>174 ± 11</td>
<td>172 ± 10</td>
</tr>
<tr>
<td>GI disorders</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak, mg/L</td>
<td>6.7 ± 0.4</td>
<td>6.6 ± 0.3</td>
</tr>
<tr>
<td>AUC, min · mg/L</td>
<td>897 ± 46</td>
<td>870 ± 37</td>
</tr>
<tr>
<td>Time to peak, min</td>
<td>172 ± 11</td>
<td>165 ± 9</td>
</tr>
</tbody>
</table>

* Significantly different from value obtained without secretin infusion: P < 0.001.

Discussion

We demonstrated in the present study that the sensitivity of the PLT for chronic pancreatitis (rate of positive results in this disease condition) can be significantly increased by administrating intravenous secretin just before ingestion of the test meal, as well as by calculating specimen-specific calibration factors obtained from the serum of each patient to determine serum fluorescein concentrations. The specificity (rate of negative results in patients without chronic pancreatitis) was not changed by these modifications. Furthermore, we found the test procedure can be simplified by taking blood samples only at 0 (basal), 120, 150, 180, and 240 min. Applying all these modifications together gave a sensitivity of the optimized PLT for chronic pancreatitis of 95% and a specificity of 81%, with positive and negative predictive values of 82% and 95%, respectively, which are close to the values reported for the secretin–cholecystokinin test (8). The complete performance of this optimized PLT is stated in Table 3.

Histology is not routinely used to diagnose chronic pancreatitis. Diagnosis of the disease is therefore indirect and based on the demonstration of either morphological or functional changes of the pancreas. Although no gold standard has been used in the present study to define pancreatic insufficiency, we assumed that an abnormal PLT result defines the presence of pancreatic dysfunction, a condition that appears commonly in chronic pancreatitis. The rate of positive results in patients with chronic pancreatitis and the rate of negative results in patients without chronic pancreatitis are thus the variables defining sensitivity and specificity of the PLT for this condition. From this point of view, chronic pancreatitis was the reference point in our study—which we defined by using imaging procedures (ERP, CT, US) and not the secretin–cholecystokinin test. First, the secretin–cholecystokinin test is not used routinely but only in special cases; second, the gold standards for diagnosis of chronic pancreatitis are imaging procedures, with ERP being the most accurate (1). Accordingly, a pathological result for the secretin–cholecystokinin test should be very carefully considered in patients with no morphological alteration of chronic pancreatitis in ERP and CT. Finally, a very high correlation has been repeatedly demonstrated between imaging findings (ERP and CT) and the secretin–cholecystokinin test (5, 6, 9). Although the use of imaging criteria for classifying patients with chronic pancreatitis may to some degree misclassify their disease status, mainly in patients with very mild or very early stages of chronic pancreatitis, this potential misclassification could only minimally affect the results obtained here for sensitivity and specificity if a diagnostic accuracy as high as 95% is accepted for ERP in chronic pancreatitis (1). To our knowledge, studies analyzing the diagnostic accuracy of imaging methods in the very early stages of chronic pancreatitis in comparison with histology are lacking; thus, the real impact of the potential misclassification of patients on the results reported here is difficult to evaluate. The use of ERP to define chronic pancreatitis is therefore an adequate standard that moreover avoids the misclassification of patients who have the disease but not exocrine pancreatic dysfunction.

In patients with a mild chronic pancreatitis, the pancreas is able to respond normally to a physiological stimulus, e.g., a meal. Indirect pancreatic function tests are thus unable to detect any abnormality in such patients (1). The initial pancreatic response to the intravenous administration of supraphysiological doses of secretin and cholecystokinin is also frequently normal in these patients but decreases if the stimulus is maintained (8). In the same way, the pancreas affected by chronic pancre-
Calculation of serum fluorescein concentration by a standard calibration factor has been routinely performed by multiplying the extinction-time curve of the test and, therefore, does not influence the result. Intravenous injection of secretin does not influence the number of patients included in this part of the study.

1. Calculation of the calibration factor (F) as follows: $F = \frac{A_i}{A_0}$, where $A_i$ is the absorbance for each sample (120-, 150-, 180-, and 240- min samples) after subtraction of $A_c$

2. Calculation of the fluorescein concentrations as follows: $[\text{fluorescein}] = F \times A_i$, where $A_i$ is the absorbance for each sample (120-, 150-, 180-, and 240-min samples) after subtraction of $A_c$

3. The result of the test is the highest serum fluorescein concentration reached (peak)

4. Normal result: peak >4.5 mg/L

### Table 3. Proposed guidelines for optimal performance of serum PLT.

<table>
<thead>
<tr>
<th>Performance of the test</th>
<th>1. Overnight fast</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. Placing of an indwelling cannula in an antecubital vein</td>
<td></td>
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<tr>
<td>3. Taking of the basal blood sample (10 mL)</td>
<td></td>
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<tr>
<td>4. Intravenous administration of secretin 1 U/kg body weight (&gt;2–3 min)</td>
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<tr>
<td>5. Ingestion of the test meal (40 g of wheat bread, 20 g of butter, 200 mL of tea) together with 1 mmol of fluorescein dilaurate spread on the bread together with the butter</td>
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<tr>
<td>6. Intravenous administration of 10 mg of metoclopramide</td>
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<tr>
<td>7. Taking of blood samples (5 mL each) at 120, 150, 180, and 240 min after test meal ingestion</td>
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</tbody>
</table>

Calculation of serum fluorescein concentration

1. Centrifugation of all blood samples to obtain serum
2. Preparation of the calibration sample by adding 60 μL of a fluorescein solution (10 mg of fluorescein disodium diluted in 100 mL of distilled water) to 1.44 mL of basal serum
3. Mixing of 500 μL of each serum (calibration serum, basal serum, and sera taken at 120 to 240 min) with 500 μL of 0.5 mol/L ethanolic KOH (each sample twice)
4. Incubation for 60 min at 70 °C
5. Cooling at room temperature
6. Addition to each sample of 1 mL of 0.15 mol/L magnesium sulfate, mixing, and centrifugation at 2671g for 10 min
7. Measurement of the supernatant photometrically at 492 nm vs water

### Calculation of serum fluorescein concentration

1. Calculation of the calibration factor (F) as follows: $F = \frac{4}{A_c - A_0}$, where $A_c$ is the mean absorbance of the calibration sample at 492 nm and $A_0$ is the mean absorbance of the basal sample

2. Subtraction of the mean absorbance for the basal sample ($A_B$) from the mean for each other sample ($A_{120}$ through $A_{240}$)

3. Calculation of the fluorescein concentrations as follows: $[\text{fluorescein}] = F \times A_i$, where $A_i$ is the absorbance for each sample (120-, 150-, 180-, and 240-min samples) after subtraction of $A_c$

4. The result of the test is the highest serum fluorescein concentration reached (peak)

5. Normal result: peak >4.5 mg/L

Pancreatitis can respond normally to the test meal given after an overnight fast in the context of the PLT and the result of the test can thus be normal. By using the intravenous infusion of secretin, we aimed at inducing a washout of the overnight-stored pancreatic enzymes, so that the test meal given immediately thereafter would require new enzyme synthesis for digestion. This new synthesis is likely to be impaired in chronic pancreatitis, as supported by our data. Among the 16 patients with chronic pancreatitis studied in the third part of the study, all patients had an abnormal PLT result, despite the small number of patients included in this part of the study. Intravenous injection of secretin does not influence the time curve of the test and, therefore, does not influence the optimal sampling time.

Calculation of serum fluorescein concentration in PLT has been routinely performed by multiplying the extinction of each sample by a standard calibration factor (2–4). This calibration factor was calculated in our laboratory by adding various amounts of fluorescein to a serum pool (final fluorescein concentrations, 0–10 mg/L) and thus constructing a calibration curve. By linear regression analysis we obtained a calibration factor of 20.83 (2). The absorbance of serum at 492 nm, however, showed a considerable intrindividual day-to-day variation as well as interindividual variation depending on the characteristics of serum (e.g., concentration of lipids) and conditions of the analysis (e.g., centrifugation, room temperature). Furthermore, the use of a calibration curve based on a wide range of fluorescein concentrations does not assure the highest accuracy of the method at the fluorescein range of 4.0–4.5 mg/L, the critical point at which a test result is defined as abnormal. We confirmed these facts in the present study and showed that the use of the same calibration factor for all patients influenced the accuracy of the PLT. This limitation can be overcome by applying a specimen-specific calibration factor for each subject each day. The fluorescein concentration of the calibration serum obtained was 4.0 mg/L, thus assuring the greatest accuracy of the test at that concentration.

The urine PLT was reported to have an overall accuracy of ~70% for detecting pancreatic dysfunction in patients with chronic pancreatitis (10–14). This accuracy was further improved to 84% by determining the fluorescein in serum (15) and introducing some test modifications (2, 3). The sensitivity of the PLT in patients with mild morphological changes of chronic pancreatitis, however, was as low as 52% (3); this sensitivity can be increased by ~30% by applying the test modifications...
described in the present study—from 46% to 75% in patients with mild chronic pancreatitis and from 71% to 100% in those with moderate disease.

Based on experience accumulated over many years, we have developed a tubeless pancreatic function test that provides a high sensitivity for chronic pancreatitis, close to that obtained by means of the invasive secretin–cholecystokinin test and higher than any other indirect test (1). This high sensitivity is, however, reached at the cost of introducing some invasive maneuvers (i.e., intravenous injection of secretin and metoclopramide, and collection of blood samples). Potential side effects of metoclopramide and secretin present very rarely after single doses. In our experience, some patients occasionally experienced a transient mouth dryness after metoclopramide administration. Few patients had nausea after secretin, which can be prevented by the slow injection of the drug (over 2–3 min). The specificity of the PLT was not affected by the modifications used. False-positive results can be obtained in patients with other pancreatic diseases (e.g., pancreatic cancer) and gastrointestinal extrapancreatic diseases that lead to maldigestion of the fluorescein dilaurate (e.g., partial gastric resection with Billroth II anastomosis, obstructive jaundice) or to malabsorption of the released fluorescein (e.g., sprue) (3, 16).

We thank E. Asche and M. Wald for skillful technical assistance.

References