Value of a Spectroscopic "Fecogram" in Determining the Etiology of Steatorrhea

Evelyne Puechant, Christine Salles, and Raymond Jensen

We have developed a “fecogram” to present the analytical results for four characteristics of fecal specimens: dry weight, total nitrogen, total fat, and hydrolyzed fat, measured by near-infrared spectroscopy. This technique gives results as precise as those of more traditional analyses for these four components. The respective correlation coefficients are 0.973, 0.960, 0.974, and 0.978. Among the digestive disorders revealed by this fecalogram is steatorrhea, which can be differentiated as being of pancreatic or intestinal etiology. The percentage of total fat that is hydrolyzed is significantly (P < 0.0001) greater in patients with malabsorption (>70%) than in those with maldigestion (<70%).

Additional Keyphrases: dry weight • total nitrogen • total fat • hydrolyzed fat • pancreatic and intestinal steatorrhea differentiated

Detection and quantification of steatorrhea are the most frequent investigations in digestive pathology. The two digestive-tract organs involved in steatorrhea are the pancreas and the intestine (1). Results of tests currently employed to detect steatorrhea are used to construct a "fecogram," on which are recorded, for the same sample, measurements of dry weight, total nitrogen, total fecal fat, and other data, according to various authors (2–4), to aid in the diagnosis of digestive deficits that lead to diarrhea. We analyze hydrolyzed fat, in addition to the three main analytes, to differentiate digestive from absorptive steatorrhea.

Common fecalogram techniques are very long (taking several hours, or several days), cumbersome, unsuited for serial analyses, and involve numerous manipulations with offensive reagents (5, 6). We present a fecalogram based on near-infrared spectroscopy (NIRA) that was first used by Maahio et al. (7) for analysis of several fecal analytes. We have adapted this technique from the chemical methods used in our laboratory to the four fecal analyses in the present fecalogram. We wanted to obtain precise results in a very short time without use of reagents or extraction, and to attempt to differentiate malabsorption from maldigestion syndromes.

Materials and Methods

Apparatus

We used a Model 450 “InfraAlyzer” (Technicon Instrument Corp., Tarrytown, NY) connected to a Hewlett-Packard Model 86 B microcomputer. The software was from Technicon, Domont, France.

Principle

The method is based on the relationship between the reflectance intensity diffused by the surface of a fecal sample at a specific wavelength and the composition of the sample (8). Each component to be measured for the fecalogram has specific absorption bands in the near-infrared, so the reflectance from the fecal sample can be related to the concentration of that component as follows:

\[ X = Z - F_1 \log R_1 - F_2 \log R_2 - \ldots - F_n \log R_n \]

where \( X \) is the concentration of the analyte, \( R_n \) is the reflectance for the filter \( n \), \( F_n \) is the scaling factor for each filter, and \( Z \) is a constant of bias correction. Scaling factors corresponding to each selected filter were calculated by multilinear regression (9) of results obtained from a training set consisting of samples analyzed by routine chemical methods and covering the whole concentration range for each analyte. The principle of the method is the following: the sum of squares of the residual values (differences between reference values and calculated values) must be as low as possible. The computer assessed every set of wavelength combinations from 1 to 19, through spectroscopic and statistical data based on a t-test where \( t = \) value of F/SD (standard deviation) value of F.

Each combination of wavelengths was characterized by the coefficient of correlation \( r \) between the chemical and the calculated values:

\[ r = \sqrt{1 - \frac{\text{SEE}^2}{\text{SD}^2(n - 1)}} \]

where \( \text{SEE} \) is the standard error of estimate, \( n \) is the number of samples, and \( k \) is the number of filters; and by the constant of Fisher (F-ratio), which indicates the quality of the regression:

\[ F\text{-ratio} = \frac{r^2(n - k - 1)}{(1 - r^2)k} \]

As the number of wavelengths in the combination increased, the correlation coefficient approached 1 and the F-ratio increased to values >50. The program was halted when there was no further increase in the two coefficients.

We tested the computer-selected regression by analyzing samples different from those used for the calibration. We compared the results with those obtained by the chemical methods routinely used in our laboratory, adjusting the NIRA results to agree more closely with those of the chemical techniques by modifying the bias constant.
Procedure

Stool specimens were collected for 72 h in plastic vessels, then analyzed without delay, or stored at -20°C until assay. The samples were obtained from the laboratory of biology of the C.R.A.M.A.\(^1\) for the controls and from patients hospitalized at the Saint-André Hospital for the pathological groups. Their daily intake of fat was limited to 80 to 100 g per day.

After stool homogenization, a sample was removed with a spatula, placed in an open cup (Technicon), and then covered by a glass slide. Exerting manual pressure on the slide kept the sample volume constant.

We then placed the cup on an interchangeable sample drawer, pushed the drawer into the NIRA spectrocope, and initiated the wavelength program. The instrument displayed the results for each analyte on the screen in less than 1 min.

Comparison Methods

The comparison routine chemical methods were the following: percentage of dry weight was obtained through measurement of the fecal water content by the Karl Fischer method (10); total nitrogen by mineralization (Berthelot’s reaction) followed by automated analysis (AutoAnalyzer II; Technicon); and hydrolyzed and neutral fecal fat by the method of Van den Kamer et al. (5).

Results

For results to be as precise as those obtained with routine chemical methods, the InfraAlyzer had first to be calibrated. The calibration results were then adapted to the chemical results. Obviously, calibration is better when the results of manual methods are accurate. However, the quality of these results also depends on the rating of the calibration set (5). Figure 1 is a histogram of residual values for hydrolyzed fat. This was obtained by using, on the abscissa, residual values associated to ranks of increasing values and, on the ordinate, plots with a known standard deviation. When the sample distribution was satisfactory, as was the case for the four analytes of the fecalogram, a gaussian plot was obtained.

Table 1 shows the best set of wavelength combinations for the different fecalogram analytes. The number of filters selected differed from one parameter to another, varying from three to seven. The greatest number of filters (i.e., seven) for obtaining a correlation coefficient of 0.973 was required for the hydrolyzed-fecal-fat regression.

The regressions of dry weight, total nitrogen, and total fat also gave very acceptable results, with correlation coefficients of 0.987, 0.974, 0.993, respectively, and very high F-ratios, exceeding 100.

The precision of the NIRA method as adapted to our chemical methods and with use of the wavelength combinations indicated in Table 2 was calculated from results obtained for 30 consecutive assays of a single fecal sample, previously analyzed by classical chemical methods. The results (Table 2) had CVs ranging from 1.19% to 2.88%.

The methods were compared by using stools from selected hospitalized patients and applying the appropriate NIRA and routine chemical techniques for each analyte. The results obtained are shown in Table 3. Linear regressions and correlation coefficients obtained in this way indicate good agreement between the methods.

Table 4 gives the results of the application of the fecalogram to stools of 40 healthy controls and 40 selected hospitalized patients suffering from steatorrhea of various etiologies. The hospitalized patients were divided into two groups. The first group consisted of 20 patients with steatorrhea resulting from intestinal insufficiency, among which were included 18 gastric resections, one Crohn’s disease, and one Whipple’s disease. The second group of 20 patients had steatorrhea due to pancreatic insufficiency, of which 16 were due to chronic pancreatitus, one to a Vater’s ampulloma, and three to cancer of the pancreas. As indicated in Table 4, wet weight was highly increased in groups I (246.1 ± 119.9 g/24 h) and II (239.3 ± 112.5 g/24 h) as compared with controls (88.63 ± 41.94 g/24 h), but was very variable in both groups. Moreover, wet weight was not significantly correlated with total fat of steatorrhea, which was sometimes greatly increased (41.7 and 48.3 g/24 h) for only slightly increased wet weights (167 and 250 g/24 h, respectively). Dry weight varied only slightly in the two groups compared with the controls, with lower (18.6 ± 4.4%) but non-significant values in gastrointestinal diseases compared with digestive diseases (20 ± 6.9%).

Total nitrogen was significantly higher in groups I (2.4 ± 0.8 g/24 h) and II (2.9 ± 1.3 g/24 h) than in controls (1.02 ± 0.28 g/24 h). Total fat concentration was also very high in groups I (17.9 ± 11.3 g/24 h) and II (15.9 ± 11.4 g/24 h) as compared with controls (2.78 ± 1.67 g/24 h), and values for hydrolyzed fat were significantly different between controls (0.83 ± 0.60 g/24 h), group I (14.8 ± 8.6 g/24 h), and group II (8.2 ± 6.9 g/24 h). The percentage of hydrolyzed fat in patients with malabsorption (group I) exceeded 70%, while those with maldigestion (group II) had less than 70% (P < 0.0001).

The correlation between concentrations of hydrolyzed fat and total fat in the 40 patients (Figure 2) shows that, for the same amount of total fat, the amount of hydrolyzed fat is greater in group I than in group II.

Discussion

The advantages of the NIRA technique over the more classical chemical methods in fecalogram analysis are obvious. The absence of any reagent, extraction, or mineralization allows this method to be applied in any laboratory, not just in specialized centers. Moreover, the rapidity of the technique (less than 1 min) allows the fecalogram to be available immediately to the clinician; thus further investigations can be implemented more promptly. Finally, a

---

\(^1\) Caisse Régionale d’Assurance Maladie d’Aquitaine, 30 avenue Charles de Gaulle, Bordeaux.
Table 1. Results of Multilinear Regression

<table>
<thead>
<tr>
<th>Wavelength, nm</th>
<th>Dry weight</th>
<th>Total N</th>
<th>Hydrolyzed fat</th>
<th>Total fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filters</td>
<td>Scaling factors</td>
<td>Wavelength, nm</td>
<td>Filters</td>
<td>Scaling factors</td>
</tr>
<tr>
<td>2330</td>
<td>6 -746.2</td>
<td>1445</td>
<td>19</td>
<td>7.747</td>
</tr>
<tr>
<td>2270</td>
<td>5 1212</td>
<td>1962</td>
<td>11</td>
<td>7.373</td>
</tr>
<tr>
<td>2330</td>
<td>6 -746.2</td>
<td>2100</td>
<td>14</td>
<td>-35.04</td>
</tr>
<tr>
<td>2270</td>
<td>5 1212</td>
<td>1962</td>
<td>11</td>
<td>7.373</td>
</tr>
</tbody>
</table>

r 0.987
F-ratio 698

Table 2. Precision of the NIRA Method

<table>
<thead>
<tr>
<th>Chemical value*</th>
<th>n</th>
<th>Dry wt, %</th>
<th>Total N</th>
<th>Hydrolyzed fat</th>
<th>Total fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>2330</td>
<td>6 -746.2</td>
<td>1445</td>
<td>19</td>
<td>7.747</td>
<td>1680</td>
</tr>
<tr>
<td>2270</td>
<td>5 1212</td>
<td>1962</td>
<td>11</td>
<td>7.373</td>
<td>2236</td>
</tr>
<tr>
<td>2330</td>
<td>6 -746.2</td>
<td>2100</td>
<td>14</td>
<td>-35.04</td>
<td>2336</td>
</tr>
<tr>
<td>2270</td>
<td>5 1212</td>
<td>1962</td>
<td>11</td>
<td>7.373</td>
<td>2236</td>
</tr>
</tbody>
</table>

* Determined by routine method. Results are mean (and SD).

Table 3. Correlation of Results by the NIRA Technique (y) and the Routine Chemical Method (x)

<table>
<thead>
<tr>
<th>Range</th>
<th>n</th>
<th>Slope</th>
<th>Intercept</th>
<th>S_y</th>
<th>r</th>
<th>x</th>
<th>y</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry wt, 40</td>
<td>0.873</td>
<td>2.764</td>
<td>1.338</td>
<td>0.973</td>
<td>6-36 (a)</td>
<td>7-35 (a)</td>
<td></td>
</tr>
<tr>
<td>Total N 30</td>
<td>0.934</td>
<td>0.058</td>
<td>0.159</td>
<td>0.960</td>
<td>0.3-2.5 (b)</td>
<td>0.4-2.6 (b)</td>
<td></td>
</tr>
<tr>
<td>Hydrolyzed fat 40</td>
<td>0.952</td>
<td>0.193</td>
<td>0.853</td>
<td>0.978</td>
<td>0.2-17.5 (b)</td>
<td>0.1-15.9 (b)</td>
<td></td>
</tr>
<tr>
<td>Total fat 40</td>
<td>0.974</td>
<td>0.246</td>
<td>1.038</td>
<td>0.974</td>
<td>0.9-18.7 (b)</td>
<td>0.2-19.2 (b)</td>
<td></td>
</tr>
</tbody>
</table>

* Results in percentage. Results in g/24 h.

Table 4. Results of Fecalograms for Patients with Gastrointestinal Diseases and Pancreatic Insufficiency

<table>
<thead>
<tr>
<th>Wet wt, g/24 h</th>
<th>Dry wt, %</th>
<th>Total N, g/24 h</th>
<th>g/24 h</th>
<th>%</th>
<th>Total fat, g/24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIRA</td>
<td>Hydrolyzed fat</td>
<td>NIRA</td>
<td>Hydrolyzed fat</td>
<td>NIRA</td>
<td>Hydrolyzed fat</td>
</tr>
<tr>
<td>Controls (n = 40)</td>
<td>30-188</td>
<td>14-24</td>
<td>1.02 ± 0.28</td>
<td>0.6-1.5</td>
<td>0.1-3.0</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>88.63 ± 41.94</td>
<td>19.12 ± 3.38</td>
<td>1.02 ± 0.28</td>
<td>0.6-1.5</td>
<td>0.1-3.0</td>
</tr>
</tbody>
</table>

Patients with gastrointestinal diseases (Group I, n = 20)

| Mean (SD) | 246.1 (199.9) | 18.6 (4.4) | 2.4 (0.8) | 14.6 (8.6) | 82.7 (7.0) | 17.9 (11.3) |
| Range | 110-681 | 9-25 | 0.9-3.7 | 5.6-31.2 | 73.9-91.2 | 6.5-48.3 |

Patients with pancreatic insufficiency (Group II, n = 20)

| Mean (SD) | 233.9 (112.8) | 20 (6.9) | 2.9 (1.3) | 8.2 (6.9) | 49.6 (12.7) | 15.9 (11.4) |
| Range | 130-615 | 10-38 | 1.0-6.0 | 2.7-25.6 | 31-66.3 | 6.5-41.7 |

comparison of the results obtained shows that the present method is as accurate as the routine chemical methods.

The NIRA method is not only of interest for analytical reasons, it also has diagnostic applications. Indeed, the detection of steatorrhea, with or without diarrhea, often requires complex complementary tests. Also the possibility of determining the etiology of steatorrhea by means of the fecalogram results alone is of great interest in diagnostic orientation. The present fecalogram, with its four components, would be of assistance.

Steatorrhea is by definition an excessive elimination of fecal fat (>5 g/24 h) (2), which may result from various hepatobiliary, pancreatic, or intestinal disorders (I, II). Among these disorders, it is important that those ascribable to intestinal malabsorption, corresponding to intestinal disease, and those due to mal digestion, resulting from pancreatic insufficiency, be differentiated (2). It is agreed that steatorrhea can be detected by measurement of fecal fat (12-14), and several tests of limited interest have been proposed to differentiate the digestive type from the absorptive type (15, 16). However, no definitive test has been established.

The present study shows that our fecalogram only requires four components (dry weight, total nitrogen, total fat, and hydrolyzed fat) for evaluating digestive function, for diagnosing steatorrhea, and for determining its etiology.

These components are important for the following reasons:
- Dry weight is indispensable for assessing digestive func-

CLINICAL CHEMISTRY, Vol. 34, No. 1, 1988
tion and, eventually, certain syndromes such as constipation and diarrhea (17, 18). However, this variable remained stable in both groups, except in certain cases of intestinal diarrhea, where it was low, and in others of fat diarrhea, where it was high.

Nitrogen is of interest because of its high increase compared with the controls, an increase that was greater in malabsorption (group II) than in malabsorption (group I), although the difference between the two groups was not very significant (P < 0.01).

Total fat assay is an indicator of steatorrhea and allows its quantification.

Data on hydrolyzed fat, compared with total fat, indicate the origin of the steatorrhea. Hydrolyzed fat corresponds to free fatty acids in stools. However, the alimentary lipids are essentially composed of triglycerides, which undergo digestion through hydrolysis, yielding mono- and diglycerides and free fatty acids (19, 20), which are absorbed by the intestinal mucosa (21). In the case of pancreatic insufficiency or malabsorption syndrome, one sees a decrease of free fatty acids, owing to an increase of triglycerides that are not hydrolyzed. In fact, our data show that patients with intestinal disease have a significantly (P = 0.0001) greater proportion of hydrolyzed fat (>70%) than do patients with digestive diseases (<70%). Our results are in harmony with those obtained by Thompson et al. (22), who observed low triglyceride concentrations in fecal samples from patients suffering from malabsorption. However, the measurement of triglycerides is very long and difficult, requiring extraction of lipids before chromatography.

The NIRA technique applied to fecalogram analysis allows determination of four major fecal components in a single sample and does not require the use of reagents, extraction, or mineralization. Furthermore, the simplicity and the rapidity of the analysis render this technique especially suitable for routine use. The clinician should therefore benefit from these improvements in the ability to assess digestive function and immediately to orient the explorations when the fecalogram reveals a steatorrhea due to a pancreatic or intestinal disorder.

We thank Dr. Beaufieux, of the laboratory of biology of the C.R.A.M.A., for obtaining fecal samples from controls.

References