Diagnostic Significance of the Sudan III Staining for Fecal Fat

OSAMU MASAMUNE, TSUNEO TAKAHASHI,* AKIO NAGASAKI, JUNJI IWABUCHI and MAKATO ISHIKAWA*

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MASAMUNE, O., TAKAHASHI, T., NAGASAKI, A., IWABUCHI, J. and ISHIKAWA, M. Diagnostic Significance of the Sudan III Staining for Fecal Fat. Tohoku J. exp. Med., 1977, 122 (4), 397-402 — To evaluate the diagnostic significance of the Sudan III staining for the fecal fat, the quantitative relationship between the number of fecal fat droplets examined microscopically and the amount of the fecal fat determined chemically was examined. In 43 out of 60 cases, the results of the chemical determination and the microscopic examination were in agreement and the accuracy was 72%. On the other hand, the false positive cases totaled 9 out of the 24 cases (37%) and 8 out of 9 false positive cases were diagnosed as showing a slight increase. In contrast, the false negative cases were only 2 out of 36 cases (6%). In addition, the comparative study of the various procedures of the Sudan III staining indicated the importance of the processing with acid and heating even in cases of steatorrhea caused by pancreatic insufficiency. ——— Sudan III staining; fecal fat; steatorrhea

The Sudan III staining for fecal fat has long been in our possession as a valuable screening procedure for the diagnosis of malabsorption syndrome. Few studies have been made, however, to clarify the quantitative relationship between the number of fat droplets detected by the Sudan III staining and the amount of fecal fat chemically determined (Drummey et al. 1961; Moore et al. 1971). Accordingly, the diagnostic significance of the Sudan III staining has remained unelucidated so far. Neither has there been any unanimity regarding its standard methods of performance or the normal range of fat droplets. It is feared, therefore, that the Sudan III staining is either misused or misinterpreted or even disregarded because of the development of other newer screening methods. The main purpose of this paper is to clarify first the quantitative relationship between the number of fecal fat droplets and the amount of fecal fat, and secondly, to re-evaluate the diagnostic significance of the Sudan III staining.

MATERIALS AND METHODS

The present study deals with 60 cases, in a comparative way, including both normal subjects and patients admitted to the Third Department of Internal Medicine with various

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degrees of malabsorption.

The solutions that were used for the Sudan III staining were (1) the saturated Sudan III solution in 95% alcohol (Solution A) and (2) 37% acetic acid (Solution B). Single drop of Solution A and single drop of Solution B, when necessary, were applied to small pieces of stool on the slide glass with the help of an applicator stick. After a thorough mixing, each specimen was heated, unless otherwise stated, and covered immediately with a cover slip. For each individual case, two specimens were produced. Three examiners were called in to count the number of fecal fat droplets using a microscopic magnification of 100, and the average value of those three readings was adopted.

Four different procedures were used in order to compare the validity of various screening methods of the Sudan III staining: (1) Method A consisted of only mixing of stool and Solution A, (2) Method B, heating the mixture in Method A, (3) Method C, mixing of stool and both Solution A and Solution B, and (4) Method D, heating the mixture in Method C. For this comparative study, 15 cases of pancreatic insufficiency were selected. The amount of fecal fat contained in the stool was chemically determined by the van de Kamer's method (1949).

**RESULTS**

As for the definition in the microscopic examination, when the number of fat droplets remained less than 10 in the field, it was regarded as normal; when the number ranged between 10 to 100, it was regarded as a slight increase and when the number was more than 100, as a definite increase. In the chemical examination, when the amount of the fat contained in 100 g of stool was less than 3 g, it was regarded as normal; when it ranged between 3 and 5 g, as a slight increase, and when it was above 5 g, as a definite increase.

Out of 24 cases in which the fat content was less than 3 g, the Sudan III staining diagnosed 15 cases as normal, 8 cases as a slight increase, and the remaining one case as a definite increase. This means that in 63% of the cases examined, the number of fat droplets found microscopically were in agreement with the degree of fat content in feces. The microscopic examination of 18 cases of a slight increase as defined by the fat content in feces also revealed that 15 cases could be classified as a slight increase, indicating that the accuracy of the microscopic examination was as high as 83%. In other 18 cases of a definite increase as defined on the basis of the amount of fat, the microscopic examination confirmed a definite increase in 13 cases, the accuracy being 72%. In total, the chemical determination and the microscopic examination were in agreement in 43 out of 60 cases and therefore the accuracy was 72%. On the other hand, the microscopically positive cases, namely, the cases diagnosed as abnormal by the Sudan III staining although diagnosed as normal by the chemical determination, totaled 9 out of the 24 cases (37%), and 8 out of 9 false positive cases were diagnosed merely as showing a slight increase. In contrast, the numbers of the false negative cases diagnosed as normal by the Sudan III staining though diagnosed as abnormal by the chemical determination, were only 2 out of 36 cases (6%) (Table 1).

Then the results obtained by applying various procedures of the Sudan III staining were compared in order to test their validity. Table 2 clearly indicates
TABLE 1. Quantitative relationship between the number of fat droplets detected by the Sudan III staining and the amount of fecal fat chemically determined

<table>
<thead>
<tr>
<th>Cases</th>
<th>Amount of the fat contained in 100 g of stool</th>
<th>Fat droplets detected by microscopic examination</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>&lt;3 g</td>
<td>15 (63%)</td>
</tr>
<tr>
<td>18</td>
<td>3~5 g</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>18</td>
<td>&gt;5 g</td>
<td>2 (11%)</td>
</tr>
</tbody>
</table>

Normal | Slight increase | Definite increase |
63%   | 33%             | 4%               |
0%    | 83%             | 17%              |
11%   | 17%             | 72%              |

TABLE 2. Comparison of the results obtained by various procedures of Sudan III staining

<table>
<thead>
<tr>
<th>Methods</th>
<th>Fat droplets detected by microscopic examination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>Method A</td>
<td>4 (27%)</td>
</tr>
<tr>
<td>Method B</td>
<td>2 (13%)</td>
</tr>
<tr>
<td>Method C</td>
<td>3 (20%)</td>
</tr>
<tr>
<td>Method D</td>
<td>4 (27%)</td>
</tr>
</tbody>
</table>

15 cases of pancreatic steatorrhea were selected for comparison. Methods A, B, C and D are as defined in METHODS.

that among those cases which were diagnosed as a definite increase by the chemical examination, 4 cases were diagnosed as normal by Method A, 2 cases by Method B, 3 cases by Method C. Method D, however, confirmed an increase in the number of fat droplet in all cases without exception.

DISCUSSION

Although there are various absorption tests to diagnose malabsorption syndromes, the standard procedure has been to measure the amount of fecal fat following the administration of a standard diet (Frazer 1955; Pimparker et al. 1961). This procedure, however, is technically cumbersome and usually requires a well-equipped laboratory which can hardly be expected in many general hospitals. Therefore, recently there is a tendency to adopt such new absorption tests as D-xylose test (Benson et al. 1957; Christiansen et al. 1959), vitamin A tolerance test (Legerton et al. 1953), Schilling test (1953), and 131I-triolein (Ruffin et al. 1956; Rivera et al. 1962), instead of measuring the amount of fecal fat, and the dependence upon the Sudan III fecal staining is becoming less and less. Under such circumstances, the present study aimed to clarify the quantitative relationship between the amount of fecal fat and the number of fecal fat droplets detected by the Sudan III staining and to re-evaluate the diagnostic significance of the
According to the definition by Drummey et al. (1961), the normal range of the number of fat droplets detected by the Sudan III staining ought to be less than 100 tiny globules of 1~4 μ in diameter with a microscopic magnification of 430. They defined the cases as a slight increase when more than 100 globules of 1~8 μ in diameter were detected, and a very definite increase when more than 100 globules of 6~75 μ in diameter were detected, respectively. Moore et al. (1971), on the other hand, set the upper limit of the normal range at 6 globules with a magnification of 100 on the basis of the results gained from normal subjects by means of the Sudan III staining. According to our own definition, the normal range should remain below 10 droplets with a magnification of 100; the specimens with 10 to 100 droplets should be categorized in the slight increase group and those with droplets above 100, in the definite increase group.

Based on this definition of grouping, we compared the results of the microscopic examination with those of the quantitative chemical determination by van de Kamer's method (1949), aiming to ascertain the relative accuracy of the microscopic examination of fecal fat droplets. Drummey et al. (1961) reported that, in 22 normal cases in which the fecal fat loss was less than 5% of the fat intake, the microscopic examination confirmed no increase of fat droplets in 86% of the assays and revealed only a slight increase in the remaining 14%. On the other hand, in cases where a minimal increase in fecal fat was detected by chemical examination, the microscopic examination diagnosed 13% of the cases as normal, while no case with more than a modest increase in fecal fat was diagnosed as normal by means of microscopic examination. Moore et al. (1971), however, asserted that the diagnostic significance of the Sudan III staining is still deniable through their comparative study of the microscopic examination and the chemical determination. They claimed that although the results of both examinations were certainly in agreement in 29 out of 42 cases, with respect to steatorrhea, a considerable number of false negative cases were detected and the accuracy was 33%; specifically in steatorrhea of a mild degree, the accuracy was only 14%.

The procedure employed by us was essentially similar to that of Moore et al. (1971). The results of our present study were as follows: when the chemical determination detected less than 3 g of fat in 100 g of stool, there was no increase in the number of fat droplets in 63% of the 24 cases examined, while 33% showed a slight increase and the remaining 4%, a definite increase. When the amount of fecal fat showed a slight increase ranging from 3 to 5 g per 100 g of stool, a slight increase in the number of fat droplets was also recognized in 83% of our cases. There was no case which remained within the normal range by microscopic examination in those cases diagnosed as a slight increase by the chemical determination. However, it should be noted that, even when there is a definite increase in the amount of fecal fat, no increase in the number of fat droplets was detected in 11% of such cases of ours.

To summarize the results obtained in the present study of a total of 60 cases,
the chemical determination and the microscopic examination were in agreement in 72% of the cases, whereas 9 (37%) out of 24 cases which were diagnosed as abnormal by the Sudan III staining were diagnosed as normal by means of the chemical determination. In sharp contrast, among 36 cases in which an increase in the amount of fecal fat was noted, only two (6%) were judged as normal by the Sudan III staining. This implies that there can be few false negative cases when the Sudan III staining is properly performed. The fact that Moore et al. (1971) found a large number of false negative cases may be explained in the following way: because they set their criterion around 15~25 μ in diameter, they may well have missed many of the globules when they counted them.

We also examined the effects of acetic acid and heating upon the results of the Sudan III staining. It is generally assumed that the Sudan III method stains only neutral fat, while the addition of acetic acid renders soap and crystalline fatty acids stainable as well. Based on this assumption, Drummey et al. (1961) emphasized that, since patients with pancreatic insufficiency tend to have neutral fat, the addition of acetic acid makes only a comparatively small difference, whereas in the cases of steatorrhea from some other causes, especially in those of intestinal steatorrhea, the simple application of the Sudan III alone seldom stains free fatty acid and therefore fails to divulge the presence of excessive fatty acid.

However, we would like to point out the great importance of the processing with acid and heating even in cases of steatorrhea caused by pancreatic insufficiency, as can be seen from Table 2. Although Moore et al. (1971) reported that the number of fat droplets does not change even by the application of either acid or heat, our study supports, without doubt, the necessity of the acid and heating procedures.

Through these findings, we conclude that, when there is an increase in the number of fat droplets detected by the Sudan III staining, the chemical determination is also highly desirable. However, as false negative cases are relatively few in the Sudan III staining, we may safely rule out the disturbance of absorption when the repeated microscopic examination showed negative results. Needless to say, in each case a careful observation is called for; a thorough mixing of the test constituents and a painstaking interpretation in accordance with the above-mentioned criteria are essential for the attainment of the desired accuracy.

References


