Application of the Seibert Tryptophane-Acid Reaction to the Serum of Malignancy

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In 1947 Seibert, Seibert, Atno, and Campbell (3) reported that the polysaccharide content of serum, as determined by the carbazole reaction of Dische, was increased in tuberculosis, the degree of increase roughly paralleling the degree of severity of the disease. In addition, the average polysaccharide content of the serum was shown to be increased in carcinoma, and in Boeck's sarcoid.

In 1948 Seibert, Pfaff, and Seibert (2), believing the significant polysaccharide to be associated with alpha-globulin, thought that it might be of a nucleic acid nature, since alpha-globulin has been shown to be increased in conditions characterized by tissue destruction. The method of Seymour Cohen (1) for the carbohydrate of desoxyribose-nucleic acid consisting of heating with perchloric acid and tryptophane, with production of a pink color, was therefore applied to serum. This reaction is more specific for desoxyribose than is the Dische carbazole reagent. In Cohen's method, desoxyribosenucleic acid is hydrolyzed by the perchloric acid, and the carbohydrate condenses with the tryptophane to give a colored compound. Cohen has shown that, although many substances under these conditions produce colored compounds with tryptophane, of the ones he studied only fructose, in comparable amounts, gives a color similar to that produced by desoxyribose.

Whether or not the color produced when the reaction is applied directly to serum is due to desoxyribose has not been determined. The color differs somewhat from that of the desoxyribose compound, and absorption spectra on dialysates from serum fail to reveal an absorption maximum at 260 m\(\mu\) (2). Fructose is not present in serum in amounts sufficient to account for the reaction (2). Seibert, Pfaff, and Seibert reported values obtained by this method on 134 patients, showing that there was a statistically significant difference between the mean values for groups of minimal, moderately advanced, and far advanced tuberculosis. In fifteen cases of carcinoma the amount of color produced was also above the average value for the normal group. The authors suggested that this test might have usefulness as a diagnostic aid in malignant disease.

Using the procedure of Seibert et al., we have studied 250 cases, as summarized in Figure 1. The results are expressed in Klett colorimeter scale readings as advocated by Seibert. The determinations were carried out as described by Seibert, i.e., to 0.25 ml. of serum in 0.75 ml. of 0.9 per cent sodium chloride were added 2 ml. of a 0.25 per cent solution of tryptophane and 3 ml. of 60 per cent perchloric acid. In place of the "serum control" used by Seibert which consisted simply of 0.25 ml. serum in 5.75 ml. of saline, we have substituted a serum control of 0.25 ml. of serum, 2.75 ml. of 0.9 per cent sodium chloride, and 3 ml. perchloric acid. This is then filtered just as are the solutions containing tryptophane in the actual determination. All tubes, both determinations and controls, were read against a blank consisting of saline and perchloric acid.

Several hundred sera have been examined by this method. The results on the 250 given in the following tables and charts are representative. We tried to be as objective as possible in doing the tests. The sera were received from the Robinson Foundation of the University of Pennsylvania, which is conducting a study of the Huggins test. The samples were in numbered tubes, and the person doing the test knew nothing at that time of the status of the patients whose sera were being studied. Huggins tests were also run on the same sera (by the Robinson Foundation Laboratory). A comparison of results by the two tests is to be reported later.

Of the 250 patients reported here, 100 had proved malignancies of various types and locations; 50 had diseases requiring hospitalization, other than cancer or tuberculosis; the remaining 100 constituted our normal group. The normal group requires further explanation: They were...

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people who came to the Cancer Detection Clinic for examination and were suffering from many disabili-

abilities—most of them minor, but including many cases of hypertension and chronic cystic mastitis. They were, therefore, not healthy, but we felt that they served our purpose better because they represented the particular age group (40–60) on which a test for cancer would be especially valuable.

Because Seibert had reported only 40 normals determined by this method, and because we had made a slight change in the method, we wished to establish our own normal range and used these 100 cases for that purpose. None showed clinical symp-
toms of malignant disease, and all had a normal Huggins test. None were hospitalized.

**RESULTS**

In Figure 1 are shown the frequency distributions, range of values, means, and the significance of differences between the means. The differences between the mean values for each of the groups are highly significant. However, the overlapping of values between the groups is so great as to make the use of the test extremely hazardous in the diagnosis of cancer. Our highest normal value was 75, which also represents the upper limit of 2σ. Anything above this value may be considered abnor-
mal, and, if this is so, then 33 per cent of the values of our malignant cases were within the normal range, and a considerably greater percentage of the values for the malignant group were within the range of the nonmalignant disease group. This means that a large percentage (33 per cent) of malignancies will give “false negative” readings.

It is also true that what may be looked upon as “false positive” values (i.e., values above 75) may be found in a certain number of supposedly normal persons. However, with this test we have found, among 200 patients examined in the Cancer De-
tection Clinic and free of clinical symptoms of cancer, only one with a serum value above 75 (the value for this serum was 76).

We have noted that our percentage of correct diagnoses in cases of malignancy has been decreasing as we have increased the number of patients studied. The same is true in a study of the Huggins test. This may be due at least in part to the fact that we are now examining a larger number of patients on the surgical service who perhaps are in a somewhat earlier stage of their disease than are many of the patients whom we see in the medical wards. Separation of the two groups of patients shows (Table 1) that the percentage of correct diagnoses in the medical group is 74 per cent, in the

![Figure 1](image_url)
surgical group 57 per cent. The correlation of the results of the test with the stage of advance of the disease is being studied further.

Agreement between the results of the Seibert test and of the Huggins test, done on the same sera, is close. The overlapping of the values for the normal, nonmalignant disease, and malignant disease groups are approximately the same for the two tests.

TABLE 1

<table>
<thead>
<tr>
<th>Patients with Malignancy</th>
<th>Seibert test over 75</th>
<th>Seibert test under 75</th>
<th>Per cent abnormal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surgical patients (44)</td>
<td>35</td>
<td>10</td>
<td>57</td>
</tr>
<tr>
<td>Medical patients (56)</td>
<td>41</td>
<td>15</td>
<td>74</td>
</tr>
</tbody>
</table>

While this work was in progress, Shetlar, Foster, Kelly, Shetlar, Bryan, and Everett (5) reported results of a test utilizing tryptophane and sulfuric acid instead of perchloric. They believed they were determining a mixture of galactose and mannose resulting from hydrolysis of a polysaccharide containing these two sugars plus glucosamine (4). The glucosamine, at the wave length used (500 mμ), was not measured to any great extent. Comparison of the Shetlar and Seibert procedures (4) showed good correlation but not absolute agreement. Use of both Seibert and Huggins tests on the same sera somewhat increases the percentage of correct diagnoses, but even when both tests are used, the diagnosis of malignancy is hazardous.

The use of this test may have a certain limited value in the Cancer Detection Clinic, where abnormal values indicate an abnormality in metabolism which should suggest a re-examination of such patients.

REFERENCES


To be published.
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