The Effect of Heat Inactivation on Precipitation of Serum Proteins by Means of Sodium Chromate in Sera of Normal and Cancerous Subjects

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The question whether the proteins in the blood serum of cancer patients differ from those of normal individuals has been the subject of numerous investigations (2, 5, 7). Various analytical procedures have been devised to demonstrate such a difference in one or another of its aspects. The results obtained in the analysis of native cancer serum are usually compared to those of native normal serum; however, an occasional method has been proposed in which determinations are made on serum in its native and in its heat-inactivated state, and the relative change in the same serum is considered. This has the advantage of eliminating to some extent individual characteristics, the absolute values of the serum being of minor importance.

The following is a summary of results obtained by the analysis of normal and cancer sera by such a method, previously described (1, 4, 6). The procedure has no claim to greater practical simplicity or to sharper differentiation between normal and cancer sera than other proposed methods. The results are reported here to describe some conclusions reached in its application which may be of use in the evaluation of other, similar procedures.

METHODS

Increasing amounts of sodium chromate in dilute propionic acid solution were added to blood serum, and the height of the precipitated protein sediment was measured in each case. This was done with native serum and simultaneously with the serum after heat inactivation. The relative position of the sedimentation zones was considered. A heat-inactivated serum is in general more stable with respect to sodium chromate than the same serum in its native state, and it thus requires a higher chromate concentration to produce a comparable degree of sedimentation. This stability difference between the native and the inactivated state is less in cancer sera and may even be completely absent.

Blood was obtained, preferably several hours after the last meal, and serum was taken off the clot after approximately 6 hours at room temperature. No preservatives were added. Two stock solutions were used: (A) 0.1 M sodium chromate (2.34 gm. Na₂CrO₄·4 H₂O with water to 100 ml.); and (B) 0.1 M propionic acid.

A series of solutions with increasing sodium chromate concentrations was made as follows: 5.0 ml. of solution A were diluted to 100 ml. with solution B. The same procedure was followed with 5.5 ml. of solution A, 6.0 ml. of A, etc., to 8.0 ml. of A.

Two rows of six flat-bottomed Pyrex tubes (7 × 100 mm.) were placed in a suitable stand in which the tubes hung vertically on their rims. In each tube was placed 0.2 ml. of the native serum. The six tubes in the rear row were gathered, covered, and placed for 30 minutes in a constant temperature water-bath at 56° C. They were then cooled and replaced in the stand.

2.0 Ml. of the appropriate sodium chromate-propionic acid solution was added to each tube. The contents were mixed gently by inverting the tubes twice. After the tubes had stood overnight (or for at least 12 hours) at 20° C., the height of the precipitate in each tube was measured (estimated to 0.1 mm.); these results were plotted on graph paper against the corresponding chromate concentration. The difference was then determined between the chromate concentration at which precipitation in the native serum began (by extrapolation to height 0) and the concentration at which sedimentation was equally strong in both sera (intersection of the plots). This expression of the difference in stability of the proteins before and after inactivation of the serum is designated d and read in mm sodium chromate per liter (for diagrams see the original article [1]).

Received for publication August 25, 1951.
The tubes should be kept scrupulously clean in order to prevent the precipitate from adhering to the sides. Between measurements they should be placed in chromic acid cleaning solution, washed, rinsed with distilled water, and dried.

RESULTS AND DISCUSSION

Physical basis of the procedure.—It has been demonstrated (3) that during heat inactivation of human blood serum a decrease of albumin content occurs, probably through adsorption of albumin on a denatured globulin complex, and that this decrease is significantly greater in cancer sera. Although the precipitation of a heat-denatured albumin-globulin mixture by an oppositely charged polyvalent ion is a complicated phenomenon, involving such insufficiently clarified factors as protective action, particle size, and hydration, it seems probable that the difference observed in the precipitation of inactivated normal and cancer serum is due to the presence of larger amounts of an albumin-globulin complex in the inactivated cancer serum which leads to heavier sedimentation at the same chromate concentrations.

Influence of temperature.—The temperature at which the sedimentation takes place is decisive. At low (refrigerator) temperatures the stability difference \( d \) is smaller in sera of cancer patients but also in sera of pregnant individuals and generally in sera of young women during the intermenstrual period of the cycle. With rising temperatures of sedimentation the stability difference of sera of cancer patients remains relatively smaller, but that of the others approaches the normal value. Typical examples of these conditions are given in Table 1.

These observations suggest that in all these conditions adsorption of a component in the protein complex occurs but that only in cancer serum are the adsorption forces sufficiently strong to prevent release of this component at higher temperatures.

The stability difference in sera of normal young women when measured at low temperature is a function of the phase of the menstrual cycle. The average values of 50 observations in 18 normal individuals are reproduced in Table 2.

Under these conditions (low temperature of sedimentation) the sera of normal young women approach the \( d \) value of "normal" serum only at the time of menstruation.

In order to exclude as much as possible divergent influences of conditions other than cancer, measurements of sedimentation were made at \( 20\degree \) C. by placing the tubes in a constant temperature cabinet maintained at that level.

Results in normal and in cancer sera.—Chart 1 represents the frequency distribution of the stability difference \( d \) at \( 20\degree \) C. in 230 presumed noncancer cases, in 126 cancer cases, and in 31 cases of pregnancy. Excluded from the noncancer cases were patients who had had recent surgery or irradiation, those known to have fever, and those known to have a positive serum Wassermann reaction, conditions in which the equilibrium of the serum proteins may be disturbed.

Cases of cancer comprised: cancer of the larynx, 4; lung, 2; palate, 1; tongue, 3; pharynx, 2; salivary gland, 1; esophagus, 1; stomach, 6; colon, 7; rectum, 4; pancreas, 3; kidney, 1; bladder, 6;

**TABLE 1**

<table>
<thead>
<tr>
<th>Temperature of sedimentation</th>
<th>20° C.</th>
<th>40° C.</th>
<th>60° C.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal woman past menopause</td>
<td>0.48</td>
<td>0.64</td>
<td>0.83</td>
</tr>
<tr>
<td>Normal woman, 12th day of cycle</td>
<td>0.18</td>
<td>0.54</td>
<td>0.80</td>
</tr>
<tr>
<td>Pregnancy, 3d month</td>
<td>0.20</td>
<td>0.54</td>
<td>0.75</td>
</tr>
<tr>
<td>Cancer of cervix</td>
<td>0.12</td>
<td>0.18</td>
<td>0.24</td>
</tr>
</tbody>
</table>

**TABLE 2**

<table>
<thead>
<tr>
<th>Day of cycle</th>
<th>1-5</th>
<th>6-10</th>
<th>11-15</th>
<th>16-20</th>
<th>21-25</th>
<th>26-30</th>
<th>over 30</th>
</tr>
</thead>
<tbody>
<tr>
<td>d in mm</td>
<td>0.28</td>
<td>0.11</td>
<td>0.08</td>
<td>0.06</td>
<td>0.21</td>
<td>0.24</td>
<td>0.38</td>
</tr>
</tbody>
</table>

prostate, 2; vulva, 5; vagina, 4; cervix uteri, 38; endometrium, 14; ovary, 11; breast, 7; skin, 2; retroperitoneal sarcoma, 1; leukemia, 1. In the cases reported here analysis was made of specimens obtained before treatment had begun, and classification was deferred until after the diagnosis had been established.

Pregnancy cases included: 1st trimester, 7; 2d trimester, 2; 3d trimester, 22 (including 2 in labor).

It is evident that the stability difference between native and inactivated serum with respect to sodium chromate is generally smaller in cancer than in normal sera, although there is some degree of overlapping. An arbitrary reference line is drawn at \( d = 0.6 \text{ mm} \) chromate per liter.

Of the four pregnancy cases which show values less than 0.6 mm, three were at term, one in the second stage of labor.

Chart 2 gives the \( d \) values for the cases of cancer of organs of the female genital tract and the breast,
separated from other organs. It is notable that 7 out of 38 cases of cancer of the cervix are found within the normal range and that the \( d \) value decreases in the order: cervix-endometrium-ovary and to generally lower levels in other internal organs. Whether this must be attributed to the difference in the nature of the tissue in which the neoplasm originates, to its connection with the circulatory systems, or, assuming that cancer of the patients had, in addition, undergone surgery. The cases of cancer of the endometrium had all been treated by surgery. The time that had elapsed

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**Chart 2.**—Frequency distribution of \( d \) at 20°C with respect to site of neoplasm.

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**Chart 1.**—Frequency distribution of \( d \) at 20°C in 280 noncancer cases, in 126 cancer cases, and in 31 cases of pregnancy.

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**Chart 3.**—Frequency distribution of \( d \) at 20°C after treatment of cancer patients.

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Cervix may on the average have been recognized at an earlier stage, to the length of time the tumor has been growing, are at present open questions. It is evident that a number of cases of cancer of the cervix were diagnosed as such, clinically, before altered protein characteristics could be detected in the blood serum by this method.

*Cancer sera after treatment.*—A number of sera were analyzed of patients who had received treatment for cancer and who were considered to be clinically well at the time the analysis was done. Chart 3 gives the results for cancer of the cervix, endometrium, ovary, and breast. In all the cases of cancer of the cervix represented here, therapy had consisted of extensive irradiation; two pa-

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It was found that 17 out of 37 cases of cancer of the cervix still showed the presence of serum proteins different from the normal. In only 1
out of 17 cases of cancer of the endometrium was such the case. No correlation could be established between the chromate value and the time that had elapsed since the conclusion of the treatment, nor between that value and the total amount of radiation administered or the histological evidence of radiation effects in vaginal smears at the time of analysis. The possibility of lymph node invasion in cancer of the cervix was raised as an explanation for the continued abnormality of the serum proteins but remains unproved. In 12 cases of cancer of the breast the results, after treatment, were similar to those of cancer of the cervix. Observations on cancer of the ovary after treatment were too few to warrant a conclusion.

SUMMARY
A method of analysis, in which the difference in stability between the proteins of native and of heat-inactivated blood serum is determined with respect to sodium chromate, was applied to specimens of 230 noncancer cases and of 126 cancer cases. It was confirmed that this difference is significantly smaller in sera of cancer patients. Consideration of the extent of the deviation from normal values in connection with the affected organs showed that the stability difference decreases in the order: cancer of the cervix—endometrium—ovary, and to generally lower values in cancer of other internal organs. Analysis of sera of cancer patients who had received treatment and were considered clinically well showed continued cancer values in 17 out of 37 cases of cancer of the cervix, as against 1 out of 17 cases of cancer of the endometrium.

ACKNOWLEDGMENTS
The author is indebted to all who have provided specimens and diagnoses. He wishes to express his thanks in particular to Dr. L. A. Emge, professor of Obstetrics and Gynecology, emeritus, for his continued interest and advice and his support of this investigation.

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