Estrone Conversion Capacity of Blood of Postmenopausal Women with Carcinoma of the Breast

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During the investigation of the fate of estrone in blood and perfused organs, it was found that estrone was converted to a biologically inactive substance (1), which could no longer be assayed as a 17-ketosteroid (2). Utilization of this latter fact in a study of the conditions under which this conversion could occur provided evidence supporting the hypothesis that an enzyme catalyzing the reaction is present in blood (2).

A number of blood samples from human female donors were found inactive in inducing the conversion. In each instance the donor had passed the menopause. Several blood samples from postmenopausal patients with carcinoma of the breast were found capable of inducing the conversion. The study to be reported was undertaken to determine grossly the extent of the divergence between normal postmenopausal women and those with carcinoma of the breast.

METHODS

Two normal young women were used as the control sources of blood. Checks on the procedure, with their blood, were requisite, because it was found that the adequacy of the White's solution as a diluent was subject to considerable variation even when freshly made. The variation was usually traced to presumed degradation of the vitamins in the White's solution. Thus, if a series of tests on specimens was run in which no estrone loss occurred, those tests were repeated using the blood from the control women at the same time to test the efficacy of the White's solution.

It was found that with these precautions the technic described below provided a sufficient degree of consistency for the study. Because of the laborious procedure, an all-or-none comparative assay technic, rather than one based on unit determination, was employed. The method in its final form was as follows:

1. Exactly 5 cc. of venous blood was diluted with 2 cc. of 0.07 M KCN solution. The resulting 7 cc. of 0.01 M KCN laked and diluted blood was thoroughly shaken while clotting proceeded. For the clinical work, the 2 cc. of 0.07 M KCN was prepared in rubber-capped vials. The blood was injected through the cap into the cyanide solution. This treatment of the blood effectively prevented growth of microorganisms, and on storage in the icebox the sample retained activity for 4-week periods.

2. One-, 0.5-, or 0.2-cc. samples of the diluted blood were added to 50 cc. of White's solution containing KCN at 0.01 M concentration (see below reasons for choice of these blood concentrations).

3. The mixture of blood and White's solution was placed in a water bath at 37.5° C. for ½ hour. Four milligrams of estrone, dissolved in 13 cc. of saline solution (4), was then added to the reaction mixture. At precisely ½ hour after the addition of the estrone, 50 cc. of chloroform was added to the vessel, and the flask was shaken violently for at least 30 minutes.

4. Ten cc. of the clear chloroform solution obtained after centrifuging the resulting emulsion was drawn off and evaporated to dryness. To the residue was added 2 cc. of ethyl alcohol. When the residue was completely dissolved, 50 cc. of 1 N NaOH was added. The resulting solution (which occasionally showed turbidity) was kept at room temperature for 5 minutes before being placed in an ice bath and made acid to phenolphthalein by the addition of CO₂.

5. After acidification the solution was extracted 4 times with ½ volume of ether. The ether was washed with H₂O to remove traces of bicarbonate and was then evaporated to a water- and ether-free residue. The residue was treated as described earlier, and the phenolic ketonic portion obtained after the Girard separation was assayed polarographically (2).

Assay criteria.—Five control runs of the experiment by the technic described above gave re-
covery values of 3.75 mg. of estrone when 4 mg. had been added to 50 cc. of White's solution as described. Because the White's solution did not contain the amount of protein which the blood added to the reaction mixture, and also to allow for the random errors inherent in routine assay procedures, a loss of 0.5 mg. out of the 4 was considered as possibly due to chance. Thus, recovery values of 3.4 mg. or less were considered as significant and indicative of the presence of estronase.

TABLE I

<table>
<thead>
<tr>
<th>Blood donor No. of donors</th>
<th>Mean age (standard deviation)</th>
<th>No. showing estronase activity</th>
<th>No. showing no measurable &quot;estronase&quot; activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>19 ± 8.7</td>
<td>3</td>
<td>16</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>14 ± 8.7</td>
<td>11</td>
<td>3</td>
</tr>
</tbody>
</table>

The volumes of 1.0, 0.5, and 0.2 cc. of diluted blood, as mentioned above, were employed, because most normal postmenopausal blood specimens contained insufficient enzyme to show an effect at a higher dilution than 0.5 cc. in 50 cc. of White's solution. Some were inactive at much higher blood concentrations. The dilutions employed as described seemed, however, to be critical for most specimens. Many specimens showed activity at 1 cc. per 50 cc., few at 0.5 cc. per 50 cc., and only one at 0.2 cc. per 50 cc.

RESULTS

The data for the comparison between normal postmenopausal women and those with carcinoma of the breast are presented in Table 1. The patients are compared at the 0.5-cc. dilution level. In the table are presented the number of patients in each group, the average age of each group, the age range, and its standard deviation. The results of the assays are presented as to both the number of patients in each group showing activity and those showing no activity.

From the results it is apparent that the postmenopausal women with carcinoma of the breast are very liable to retain in their blood a capacity to convert estrone which normally should disappear at the menopause. The relationship between this finding and the now well recognized therapeutic value of estrogens in inhibiting the growth of this particular malignancy should prove of interest.

SUMMARY

A semi-quantitative test of the estronase concentration in human blood has been described. The test was employed in a comparison of the titer of normal postmenopausal blood and that of postmenopausal women with carcinoma of the breast. The data show that the postmenopausal women with carcinoma of the breast can be expected to have a significantly higher blood titer of the enzyme than do normal postmenopausal women.

ACKNOWLEDGMENTS

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REFERENCES

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