It is found that the general direction or the slope of the curve is important. It is obtained from the linear regression of data. Based on the positive or negative value of the slope, the proper equation for the polynomial curve results in an improvement. Polynomial equations of the second order, \( x = a_0 + a_1x + a_2x^2 \) and \( x = a_0 + a_1y + a_2y^2 \) are compared in Table 1. For a positive slope, the equation \( x = a_0 + a_1y + a_2y^2 \) results in a better correlation. For a negative slope, the equation \( x = a_0 + a_1y + a_2y^2 \) results in a better correlation. A majority of radioimmunoassay tests follow the latter equation.

The index of correlation is obtained from the following equation (4):

\[
r = \sqrt{1 - \frac{(n - 1) \times (x - x_m)^2}{(n - 3) \times (x - x_m)^2}}
\]

where \( x_e \) is the estimated value and \( x_m \) is the average value.

References

Effect of Temperature and Cortisol on the Distribution of Endogenous Aldosterone in Blood

To the Editor:
When tritium-labeled aldosterone is incubated in whole blood in vitro, the distribution of the labeled aldosterone between the erythrocytes and the plasma is a function of the temperature of the blood and the concentration of cortisol in the blood. As the temperature of the blood is increased from 4 to 37 °C, the ratio of tritium-labeled aldosterone in erythrocytes to that in plasma increases from 0.2 to 0.7 (1). This effect of temperature is most pronounced when cortisol concentrations are low. To determine if the temperature at which blood samples are processed altered the results of our assay for plasma aldosterone, we compared concentrations of endogenous aldosterone in plasma from blood processed at 4 °C to those in plasma from blood processed at 25 °C. We also determined to what extent the concentration of endogenous cortisol influenced the effect of temperature on the distribution of endogenous aldosterone in blood.

Blood samples were obtained from 10 normal subjects, ages 22-36 years, who gave informed consent and were not taking medications. At 0800 h on day 1 of the study, 80 ml of blood was obtained (normal-cortisol sample). The subjects then received, orally, 0.5 mg of dexamethasone at 1800 h, 2300 h, and at 0700 h the next day. Ninety minutes after the third dose of dexamethasone another 60 ml of blood was obtained (low-cortisol sample). The subjects then received orally 60 mg of cortisol, and 2 h later a third 60-ml sample of blood was withdrawn (high-cortisol sample).

Each blood sample was immediately divided between two tubes which contained 1 ml of an anticoagulant solution consisting of disodium EDTA (38 g/liter) in 0.15 mmol/liter sodium chloride solution. One of the duplicate tubes was pre-chilled, and after the blood was added it was placed in crushed ice for 30 min. The other tube of blood was left setting at room temperature, 25 °C, for 30 min. The chilled blood sample was then centrifuged at 4 °C and the non-chilled blood sample was centrifuged at room temperature. Aldosterone was determined by radioimmunoassay (2) and fluorogenic steroids by the Mattingsly technique (3).

The concentrations of fluorogenic steroids were the same in plasma obtained from blood processed at 4 °C and 25 °C. The range of cortisol concentrations was 70-270 µg/liter in the normal cortisol samples, 50-60 µg/liter in the low-cortisol samples and 250-300 µg/liter in the high-cortisol samples. The temperature at which the blood samples were processed did alter the amount of endogenous aldosterone in the plasma.

<table>
<thead>
<tr>
<th>Test</th>
<th>Slope</th>
<th>Index of correlation</th>
<th>( x = a_0 + a_1x + a_2x^2 )</th>
<th>( x = a_0 + a_1y + a_2y^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triiodothyronine</td>
<td>positive</td>
<td>0.9974</td>
<td>0.9970</td>
<td></td>
</tr>
<tr>
<td>Thyrotropin</td>
<td>positive</td>
<td>0.9990</td>
<td>0.9769</td>
<td></td>
</tr>
<tr>
<td>Thyroxine</td>
<td>negative</td>
<td>0.9955</td>
<td>0.9989</td>
<td></td>
</tr>
<tr>
<td>Digoxin</td>
<td>negative</td>
<td>0.9979</td>
<td>0.9991</td>
<td></td>
</tr>
<tr>
<td>Vitamin B₁₂</td>
<td>negative</td>
<td>0.9523</td>
<td>0.9992</td>
<td></td>
</tr>
<tr>
<td>Folate</td>
<td>negative</td>
<td>0.9112</td>
<td>0.9897</td>
<td></td>
</tr>
</tbody>
</table>


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PLASMA ALDOSTERONE

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Aldosterone, ng/liter*</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 °C</td>
<td>195 ± 36 232 ± 44b</td>
</tr>
<tr>
<td>4 °C</td>
<td>267 ± 48 298 ± 52c</td>
</tr>
<tr>
<td>High-cortisol samples</td>
<td>144 ± 21 151 ± 26a</td>
</tr>
</tbody>
</table>

* Mean ± SEM. b Level in 25 °C plasma vs. 4 °C plasma P < 0.01. c Level in 25 °C plasma vs. 4 °C plasma P < 0.02.

Thus, aldosterone concentrations were significantly higher in plasma obtained from blood processed at 4 °C.
than in plasma processed at 25 °C when cortisol concentrations were normal or low. When cortisol concentrations were high, however, the temperature at which the blood was processed did not affect the concentration of aldosterone in plasma. The magnitude of the differences in plasma aldosterone, however, are less than the magnitude one might have expected from the previous studies. Chavarri et al. (1) found that when plasma cortisol concentration was very low the erythrocyte/plasma ratio of tritium-labeled aldosterone concentration was 58% lower at 4 °C than at 20 °C (1). The results of this study show that the temperature at which blood is processed has only a minor effect on the distribution of endogenous aldosterone in blood when cortisol concentrations are normal or low and no effect when cortisol is above-normal. The average difference was 10% in the low-cortisol samples, 15% in the normal-cortisol samples.

We conclude that the effect of temperature on the distribution of endogenous aldosterone in blood will probably be of little clinical importance in most situations. However, when plasma cortisol concentrations are normal or low and one is attempting to detect small differences in aldosterone, the effect of temperature on the distribution of aldosterone must be taken into account.

References

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