EFFECT OF DIALYSIS AND OXIDATION ON PLASMA PARATHYROID HORMONE ACTIVITY

By
Leo E. Reichert Jr. and Maurice V. L'Heureux

ABSTRACT

Comparative studies are reported on the effect of dialysis and peroxide oxidation upon the calcium mobilizing activity of a potent parathyroid gland preparation, Injection Parathyroid, U.S.P. (Lilly), when in aqueous solution and when incubated with freshly collected rat plasma prior to treatment. It was found that although dialysis and oxidation markedly decreased the potency of the parathyroid gland extract employed, prior incubation with rat plasma prevented this effect from occurring. It is suggested that a binding of the active principle of the extract to plasma proteins could account for the phenomena observed. The suggestion that oxidative inactivation of parathyroid hormone is of significance, in vivo, would not seem to be in accord with the observations recorded here.

In recent years there has been a renewal of interest in the chemistry and physiology of the parathyroid hormone. Important advances have been made in its purification (Rasmussen 1957; Munson 1959; Rasmussen & Craig 1959; Aurbach 1959) and new concepts have been introduced to explain its mechanism of action on the molecular level (Neuman & Neuman 1958; Firschein et al. 1959). Few advances, however, have been made in our understanding of the parathyroid hormone as it exists in the circulation. Recently, Reichert & L'Heureux (1960 a; 1961, in press) have observed exogenous parathyroid activity to be associated with an alpha globulin and with an albumin fraction.

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of rat plasma, and with a beta and gamma globulin fraction of human plasma (Cohn Fraction II–III). They also detected endogenous parathyroid hormone like activity in the beta-gamma globulin fraction of normal human plasma, and estimated the level of circulating endogenous activity to be of the order of 40 U.S.P. units of activity per 100 ml of plasma.

Reports in the literature have suggested the active principle of parathyroid gland extracts to be dialyzable and ultrafilterable (Kenny et al. 1956; Davies & Gordon 1954; Rasmussen & Westfall 1957). Rasmussen (1958) has observed parathormone »B« to be inactivated following treatment with hydrogen peroxide and reactivated following reduction with cysteine. He suggested that this may be of significance in vivo.

In this report the effects of dialysis and peroxide oxidation upon the activity of various parathyroid extract-plasma preparations were examined in an effort to further extend our information on the characteristics of plasma parathyroid hormone activity.

**METHODS**

The calcium mobilizing activity of parathyroid extract and various parathyroid extract-plasma preparations were estimated as previously described (Reichert & L'Heureux 1960 b). In brief, the assessments of potency are made from the increase in serum calcium induced by 5 ml of a test preparation in fasted, parathyroidectomized female rats, weighing 120 ± 10 grams, 6 hours after its intraperitoneal administration. Serum calcium is determined by the method of Natelson & Peniall (1955).

Dialysis of the various preparations were performed against cold distilled water through use of an Oxford Model B. Revolving Multiple Dialyzer. All dialysis were carried out in the cold (2–4 degrees) for 24 hours with changes of dialysate at 3, 6 and 9 hours. Cellulose tubing (A. H. Thomas No. 4465-A2) was employed as the dialyzing membrane.

Peroxide oxidation of the various preparations was carried out according to the method of Rasmussen (1958). The test preparations were adjusted to 0.1 m with 30 % hydrogen peroxide, and the oxidation terminated after 30 minutes incubation at room temperature by the addition of 1.0 mg of catalase.

Incubation of whole or lyophilized parathyroid extract with plasma preparations was allowed to proceed for one hour at 37.5°C with slow stirring over a magnetic stirrer.

»Hormonally active plasma« was the term applied to rat plasma containing detectable levels of parathyroid hormone activity. Hormonally active rat plasma was prepared as follows: 500 U.S.P. units of parathyroid extract were injected I.P. into normal female rats weighing about 200 grams. These animals were then exsanguinated after one hour, and the plasma collected at the centrifuge. A 5 ml aliquot of plasma prepared in this manner was found to possess detectable levels of parathyroid activity; that is, was able to increase the serum calcium levels of the assay animal by 1.5 ± 0.5 mg % (Table 3).

The source of parathyroid hormone employed in this work was »Injection Parathyroid, U.S.P.« manufactured by Eli Lilly and Co., Indianapolis, Indiana, assigned a potency of at least 100 U.S.P. units per ml by the manufacturer.
RESULTS

Table 1 outlines the results obtained when various doses of parathyroid hormone activity were administered to the assay animal in 5 ml of water or in 5 ml of freshly collected, normal rat plasma. As can be seen, the plasma preparation did not significantly alter the sensitivity of the assay system.

When preparations containing 50 and 100 U.S.P. units of parathyroid hormone activity per 5 ml of distilled water were dialyzed for 24 hours in the cold as previously outlined, the biological activity was sharply reduced (Table 2). In order to examine the effect of normal plasma upon this inactivation, sufficient lyophilized parathyroid extract was dissolved in freshly collected rat plasma.

Table 1.
Effect of injection medium upon sensitivity of the assay system.

<table>
<thead>
<tr>
<th>Injection medium (5 ml)</th>
<th>Number of animals</th>
<th>Units of activity present</th>
<th>Change in serum calcium, mg %/o Mean ± Std. Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>9</td>
<td>25</td>
<td>1.1 ± 0.3</td>
</tr>
<tr>
<td>Distilled water</td>
<td>10</td>
<td>50</td>
<td>1.9 ± 0.3</td>
</tr>
<tr>
<td>Distilled water</td>
<td>10</td>
<td>100</td>
<td>2.7 ± 0.5</td>
</tr>
<tr>
<td>Plasma</td>
<td>10</td>
<td>25</td>
<td>1.7 ± 0.3</td>
</tr>
<tr>
<td>Plasma</td>
<td>10</td>
<td>50</td>
<td>2.5 ± 0.5</td>
</tr>
</tbody>
</table>

Table 2.
Effect of dialysis and oxidation upon biological activity of parathyroid extract in water and after incubation with rat plasma.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Injection medium (5 ml)</th>
<th>Number of animals</th>
<th>Units of activity present</th>
<th>Change in serum calcium, mg %/o Mean ± Std. Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dialysis</td>
<td>Distilled water</td>
<td>16</td>
<td>50</td>
<td>0.3 ± 0.3</td>
</tr>
<tr>
<td>Dialysis</td>
<td>Distilled water</td>
<td>5</td>
<td>100</td>
<td>0.0 ± 0.2</td>
</tr>
<tr>
<td>Dialysis</td>
<td>Plasma</td>
<td>6</td>
<td>50</td>
<td>1.6 ± 0.2</td>
</tr>
<tr>
<td>Dialysis</td>
<td>Plasma</td>
<td>6</td>
<td>100</td>
<td>2.4 ± 0.6</td>
</tr>
<tr>
<td>Oxidation</td>
<td>Distilled water</td>
<td>5</td>
<td>50</td>
<td>0.2 ± 0.2</td>
</tr>
<tr>
<td>Oxidation</td>
<td>Distilled water</td>
<td>5</td>
<td>100</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>Oxidation</td>
<td>Plasma</td>
<td>5</td>
<td>50</td>
<td>1.7 ± 0.1</td>
</tr>
<tr>
<td>Oxidation</td>
<td>Plasma</td>
<td>5</td>
<td>100</td>
<td>2.5 ± 0.2</td>
</tr>
</tbody>
</table>
Table 3.
Effect of dialysis and oxidation on hormonally active rat plasma.*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of animals</th>
<th>Change in serum calcium Mean ± Std. Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>10</td>
<td>1.5 ± 0.5</td>
</tr>
<tr>
<td>Dialysis</td>
<td>5</td>
<td>1.4 ± 0.3</td>
</tr>
<tr>
<td>Oxidation</td>
<td>5</td>
<td>1.3 ± 0.1</td>
</tr>
</tbody>
</table>

* All injections consisted of 5 ml of plasma.

plasma to give a final preparation with an activity of 50 or 100 U. S. P. units per 5 ml of solution. These mixtures were then allowed to incubate at 37.5° C for one hour, after which they were dialyzed exactly as previously described. As can be seen from Table 2, dialysis did not affect the potency of the preparation following incubation with plasma.

Next, hormonally active plasma was prepared and again dialyzed in the same fashion. No marked decrease in biological activity was observed in this preparation subsequent to dialysis (Table 3).

A similar sequence of experiments were performed in which various parathyroid extract-plasma preparations were subjected to peroxide oxidation. When solutions of parathyroid extract containing a specific activity of 50 or 100 U. S. P. units per 5 ml of solution were treated with hydrogen peroxide as described, a dramatic decrease in biological activity was observed (Table 2). When, however, lyophilized parathyroid extract was added to 5 ml of freshly collected rat plasma and allowed to incubate at 37.5° C for one hour prior to oxidation, no such decreased biological activity was observed (Table 2). Similarly, the calcium mobilizing potency of hormonally active plasma was also not affected by this peroxide oxidation (Table 3).

D I S C U S S I O N

The data seem to indicate that the calcium mobilizing activity of a potent parathyroid extract, Injection Parathyroid, U. S. P. (Lilly) is markedly decreased subsequent to dialyses against distilled water, and oxidation with hydrogen peroxide. When the same parathyroid extract is incubated with plasma prior to dialysis or oxidation, this phenomena was not observed to occur. Rasmussen (1959) has suggested that a cofactor, perhaps a metal ion, might be required for hormonal activity. Also, as previously indicated, there are reports in the literature to suggest that the active principle of parathyroid
gland extracts is capable of passing through dialyzing membranes. Oxidation of a metal ion factor, or passage of the active principle through the dialyzing membrane could, of course, adequately explain the loss of activity noted subsequent to these procedures. What appears of significance here, however, is the »protective effect« afforded by plasma. The data presented would seem to suggest such a protective effect, and its nature is, of course, of considerable potential importance. A possible explanation in this connection would be to postulate a binding of the active moiety of the extract to a plasma protein. Such a situation would not be totally unexpected. A binding of this type could prevent the loss of activity upon dialysis by simply preventing the active hormonal moiety from passing through the dialyzing membrane and could also conceivably prevent loss of activity upon oxidation by either sterically protecting an »active site« on the hormone, or preventing easy oxidation of any possible required cofactor. The protective effect exhibited against oxidative inactivation by plasma would seem to run counter to the possibility of oxidation-reduction being of significance in vivo, as has been suggested (Rasmussen 1958). The results seem to indicate a dynamic relationship between the biological activity of parathyroid gland extract and plasma proteins.

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REFERENCES

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