Stabilization and Preservation of Serum Prostatic Acid Phosphatase Activity

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The effects of pH and temperature of storage on the stability of the prostatic fraction of serum acid phosphatase activity have been studied in order to provide a satisfactory method for the preservation of activity in sera shipped from other hospitals. Addition of citrate tablets of a composition to buffer the sera at pH 6.2 was found to preserve the original activity at 25° for at least 7 days. These results were validated on a series of sera, subdivided into control and citrate-containing aliquots, shipped from the participating hospitals.

The measurement of the prostatic fraction of serum acid phosphatase activity is a valuable laboratory adjunct in the elevation of prostatic cancer. Elevations of this fraction are generally considered to be indicative of metastatic extension beyond the limits of the gland (1). Reduction of this elevated enzyme activity is one of the objective responses used in gauging the effectiveness of treatment. Thus, it is of considerable importance that the conditions of collection, transport, and storage of the serum prevent any deterioration of the enzyme activity. The instability of this enzyme activity in serum was recognized early (2). However, no systematic study of conditions favoring its preservation under the circumstances of a long-term study requiring shipment of

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samples is available. The results of such a study are reported here, and a successful and simple method for preservation of serum prostatic acid phosphatase activity is described.

**Materials and Methods**

Serum total and prostatic acid phosphatase were measured by a modification of the method of Fishman and Lerner (3). Blood samples were collected in the morning, allowed to clot at room temperature for 1 hr., and the serum removed. For the pH preservation studies, an aliquot was treated immediately, and both the treated and untreated samples were assayed. Samples were stored at 25°, 5°, and -15°, and aliquots were assayed daily for 2 weeks. Samples from normal controls, urology patients without cancer, and patients who had prostatic cancer with normal or elevated enzyme levels were studied. Finally, samples with and without added preservative (disodium citrate) were shipped from cooperating Veterans Administration hospitals for assay.

**Results**

Since the control of either pH or temperature of storage have been reported to be of primary importance for the preservation of the serum acid phosphatase activity (2, 4), these variables were studied systemati-
cally.

The effects of pH and short-term storage at 25° were first examined by adjustment of aliquots of a high-activity serum sample to various pH's covering the range from 4 to 8 with acetic acid. The samples were allowed to stand 24 hr. at 25° and then assayed (Fig. 1). The activity was equal to or greater than the original activity in the pH 5–7 range. A marked decrease was evident at pH 8 and somewhat less so at pH 4. These studies were then extended to 3 storage temperatures and 3 days (Fig. 2). The deleterious effect of pH 4 was evident at all temperatures, with observation of a consistent decrease at 25° and 5°, and complete loss of activity following freezing at this pH. At pH 8, the loss of activity was complete by 3 days at 25°, whereas cooling or freezing these samples greatly retarded this loss. It should be emphasized that serum is normally at pH 8.0–8.5 because of loss of CO₂. There was evident a small but consistent increase in activity around pH 6.

These studies were then repeated and extended with the use of citrate because of the great potential convenience of using a solid buffer prepared in a preweighed tablet form as opposed to shipment with freezing or refrigeration. Maximum activity was again found around pH 6. A

*Utilizing phenyl phosphate substrate, pH 4.9, with aminoantipyrine color development of the released phenol.
tablet containing 18 mg. of disodium citrate yielding a pH of 6.2 when added in the proportion of 1 tablet per milliliter of serum was then used for the studies depicted in Fig. 3–8. Figures 3–5 show the means of results obtained with 14 normal serums studied over 7 days with storage at 25°, 5°, and −15°. It is evident that pH adjustment with citrate not only preserved the original activity of the prostatic fraction but resulted in a significant (p < .02) increase at all temperatures of storage by Day 2. The activity of the nonprostatic fraction with citrate declined slowly, but the decrease was significant by Day 1 (p < .02). The control samples,

![Fig. 1. Effect of serum pH adjustment with acetic acid on prostatic acid phosphatase activity. Adjusted samples were stored 1 day at 25° and then assayed. Activity of freshly drawn blood was 71.4 K-AU./100 ml. serum.](image)

![Fig. 2. Effects of pH of sample, temperature of storage, and time of storage on prostatic acid phosphatase activity. Adjustment of pH was with acetic acid.](image)
Fig. 3. Preservation of serum acid phosphatase activity by citrate at 25°; 14 normal serums assayed daily for 1 week. Declines in both total and prostatic fractions without citrate were significant at Day 1 (p < .01), whereas increase in tartrate-inhibitable fraction with citrate was significant at Day 2 (p < .02). Fig. 4. Preservation of serum acid phosphatase activity by citrate with storage at 5°; 14 normal serums assayed daily for 1 week. Fig. 5. Preservation of serum acid phosphatase activity by citrate with storage at -15°; 14 normal serums assayed daily for 1 week. No statistically significant deterioration occurred in any samples in first week.
Fig. 6. Preservation of serum acid phosphatase activity by citrate at 25°; 5 serums with elevated activity assayed daily for 1 week. Significant deterioration occurred in both total and tartrate-inhibitable fractions at Day 1 (p < .05), whereas prostatic fraction increased significantly (p < .05) by Day 2. Fig. 7. Preservation of serum acid phosphatase activity by citrate with storage at 5°; 5 serums with elevated activity assayed daily for 1 week. No significant deterioration occurred. Fig. 8. Preservation of serum acid phosphatase activity by storage at −15° and effect of citrate; 5 serums with elevated activity assayed daily for 1 week. No deterioration found with or without citrate.
containing no citrate, rapidly lost activity at 25°, and although stable at the lower temperatures (as compared to the initial measurements) did not evidence the increase observed with citrate. The control non-prostatic fraction declined rapidly at 25° and 5°, and more slowly at −15°. The decrease was significant (p < .01) by Day 1 for both the total acid phosphatase control and the prostatic fraction control at 25°.

The results obtained with high activity serums from patients with cancer of the prostate are shown in Fig. 6–8. The protective effect of pH adjustment with citrate was again most evident at 25°, with only small additional preservation apparent at 5° and −15°. At these high levels of activity, cooling the samples afforded similar preservation. Without the addition of citrate, the total activity and the prostatic fraction underwent a significant drop at 25° on Day 1 (p < .05), whereas with citrate the slight but consistent increase in the prostatic fraction was significant by Day 2 (p < .05).

The effectiveness of the citrate preservation has been tested by assaying a series of serums shipped from cooperating Veterans Administration hospitals. The freshly drawn serums were divided into 2 portions and citrate added to 1, the other serving as a control. As shown in Table 1, there was significantly less activity in the control aliquots without added citrate. Since all of the samples in this series were received between November and March, it may be anticipated that even greater losses would be encountered in the summer months.

**Discussion**

The effects of pH and temperature on the stability of prostatic tissue acid phosphatase were first noted by Kutscher and Wolbergs (5) who originally described the enzyme (6). Herbert (2) extended these studies as a part of an effort to increase the specificity of the serum prostatic acid phosphatase assay. She found the serum enzyme to be inactivated

<p>| <strong>Table 1. Preservation of Serum Prostatic Acid Phosphatase by Citrate in Shipped Samples</strong> |
|---------------------------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th><strong>Group</strong></th>
<th><strong>No.</strong></th>
<th><strong>Mean (K.A.U./100 ml.)</strong></th>
<th><strong>With citrate</strong></th>
<th><strong>Without citrate</strong></th>
<th><strong>p</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonecancer patients</td>
<td>24</td>
<td>0.4</td>
<td>0.3</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Cancer patients with normal acid phosphatase</td>
<td>15</td>
<td>0.4</td>
<td>0.3</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>Cancer patients with high acid phosphatase</td>
<td>52</td>
<td>67.2</td>
<td>57.8</td>
<td>&lt;0.01</td>
<td></td>
</tr>
</tbody>
</table>

*Samples shipped by airmail, Special Delivery, to Minneapolis from Iowa, Kentucky, Kansas, California, Maryland, New Jersey, Oregon, and Georgia. They were received from 1 to 5 days after date blood was drawn (November to March).*
50% in 10 min. at 37° in pH 7.4 acetate buffer, whereas at pH 4.8 there was less destruction. Woodard (4) reported the same phenomenon using β-glycerophosphate as substrate, and observed that the enzyme in liver and prostate behaved in a similar fashion. She stated that essentially complete protection was afforded at pH 6 or lower or simply by refrigeration. Davison (7) assayed a single specimen 4 times over a 4-month period with storage at −20° and reported no loss of activity. London et al. (8) used a partially purified enzyme from prostatic tissue to study the kinetics of heat denaturation and found the pH and the nature of the buffer anion to be significant factors. The rate of denaturation in acetate buffer was concentration-dependent, increasing with increasing concentration. The destruction was only 10% in 24 hr. at 50.3° in 0.045M acetate, pH 5.0. The rate observed with citrate was higher under these conditions but would be insignificant at lower temperatures as reflected by an estimated ΔE° of 92 kcal./mole. London et al. (9) then extended and applied these studies to the serum enzyme in vitro and in vivo. They found the in-vitro half-life in whole serum at 37° to vary between 1.3 and 5.0 hr. (mean about 2.5 hr.) in individual serum. They also presented data from patients with prostatic cancer indicating that level of serum activity might be changed inversely with body temperature. No attention was directed to conditions providing preservation of the serum enzyme activity beyond the day of blood collection. Anagnostopoulos (10) studied a partially purified preparation of the prostate enzyme and consistently found that citrate activated the enzyme and protected it from the action of a variety of inhibitors, including phosphate and fluoride. No study of the serum enzyme was made.

There has been no further extension of the basic data outlined above directed at the problem of preservation or stabilization of the enzyme activity as found in whole serum. Studies of tissue extracts have continued to appear (11–13) in the effort to elucidate mechanisms, but the results are not pertinent to the present problem, except to establish that citrate has a definite, pH-dependent, protective (or activating) effect on the acid phosphatase contained in these preparations.

The results of the present studies are consistent with the previous work. They also provide data demonstrating that citrate buffer, pH 6.2, is adequate for preservation of the endogenous prostatic acid phosphatase activity of whole serum for extended periods of time at ambient temperature.

References