Measurement of Prostatic Acid Phosphatase in Serum and Bone Marrow: Radioimmunoassay and Enzymic Measurement Compared

Peter R. Huber,1 André Scholer,2 Elisabeth Linder,1 Volker Hagmaler,3 Hans Vogt,3 Peter Christen,3 Urs Eppenberger,1 and Georg Rutishauser3

We quantitated the concentrations of prostatic acid phosphatase (EC 3.1.3.2) in serum and bone-marrow aspirates with three commercial radioimmunoassay kits, and the catalytic activities with a thymolphthalein monophosphate-based enzyme test. The enzyme’s immunological activity in serum was compared with its catalytic activity for its potential as a detector of early prostatic cancer and its performance as an early marker of metastatic activity in bone. Neither measurement is useful for detecting early stages of prostatic cancer. The spread of carcinoma to lymph nodes or to bone is detected with greater frequency by radioimmunoassay than by the enzymic test. Radioimmunoassay also detected metastasis to the bone more frequently than did physical methods. Analytical and clinical performance of the four methods is described.

**Additional Keyphrases:** enzyme activity • cancer • catalytic vs immunoreactivity of enzymes • clinical laboratory diagnosis of prostatic cancer • "kit" methods • scintigraphy • thymolphthalein monophosphate as substrate

Carcinoma of the prostate in men older than 40 is still a major cause of death from cancer. Because early detection is necessary for successful management of this disease, clinicians would like to be able to rely on one or more biological “markers” to detect the presence of prostatic cancer.

Besides digital examination of the prostate, the oldest and most popular diagnostic aid is the measurement of prostatic acid phosphatase (PAP; EC 3.1.3.2).4 Measurement of this enzyme’s activity in the diagnosis of prostatic carcinomas, first proposed by Gutman et al. (1), has certain drawbacks, depending on which substrate or inhibitor is used to discriminate between acid phosphatases derived from sources other than the prostate. Use of immunological techniques, especially radioimmunoassays (RIA), held out the prospect of improving specificity and eliminating problems related to the lack of stability of the enzyme’s activity. Reports on the application of the immunological detection of PAP for the early recognition of prostatic carcinoma by Foti et al. (e.g., 2) caught the attention of many workers in the field and prompted them to apply RIA to the measurement of PAP.

Following the same line of thinking, we considered whether we should replace the well-established and relatively inexpensive determination of enzymic activity, in which thymolphthalein monophosphate is used as substrate (3), with a test based on radioimmunological technology. Our first experiments, carried out with an early version of the PAP-RIA test introduced by New England Nuclear, were very disappointing (4), however, and we decided to test other available kits as well.

As became evident in the course of the experiments, it is very difficult to compare test results obtained with the different methods. We therefore applied the method of the “inverse distribution function” (IDF) (5) to represent data in such a way that results for different concentrations of PAP could be compared. From these IDF plots we could determine additional information on the proportions of true positives, false positives, true negatives, and false negatives (6).

Because it is of such great importance to distinguish between benign prostatic lesions and early stages of carcinoma rather than between normal tissue and carcinoma in general, we wanted to establish the precision of values in the concentration range of PAP characteristic for these two pathological situations. The course of therapy of prostatic cancer depends greatly on information as to the presence or absence of metastasis to the bone, so we extended our studies of serum to bone-marrow aspirates.

**Materials and Methods**

**RIA Kits Used, and Their Characteristics**

**Kits.** We tested three commercially available kits for the radioimmunological determination of prostatic acid phosphatase, those of Clinical Assays, Travenol, Cambridge, MA 02139; Byk-Mallinckrodt, Inc., St. Louis, MO 63134; and New England Nuclear, Dreieichenheim, F.R.G. In parallel, we used an in-house enzyme test for prostatic acid phosphatase with thymolphthalein monophosphate (3) as substrate, run on a continuous-flow analyzer (SMAC; Technicon Instruments Corp., Tarrytown, NY 10591).

**NEN:**
- Standards and tracer (2.2 μCi) are the no. 2 isoenzyme (PAP), source and purification procedure unspecified
- Antiserum: raised in rabbits against PAP of unspecified source
- Cross reaction: isoenzymes 5 and 3 cross react by 1%
- An overnight and a 4-h procedure are offered, combined with sequential incubation
- Standard range: 1–50 μg/L (six standards)
- Reagents provided in lyophilized form

**Mallinkrodt:**
- Standard and tracer (no indication of activity) of unspecified source, in stabilized human serum
- Lyophilized reagents are provided
- Antiserum: raised in rabbits or goats and lyophilized
- Cross reaction: we found erythrocyte acid phosphatase had no effect on the common assay procedure
- An overnight and a 4-h procedure are offered, along with sequential incubation
- Standard range: 1–40 μg/L (six standards)

**Clinical Assays:**
- Standard and tracer (1 μCi) are originally from seminal fluid and purified according to the method of van Etten and Saini (7)
- Antiserum: raised in goats
• Cross reaction: we detected none with acid phosphatases extracted from erythrocytes or platelets
• An overnight procedure is offered, along with a one-day procedure that requires more serum (sequential procedure)
• Standard range: 1–30 μg/L (four standards)
• High stability of reagents guaranteed by manufacturer over long period. Ready-to-use reagents; no errors on dissolving lyophilisates

To increase the number of samples assayed per run, we halved the specified amount of all reagents and of sample. To obtain the same counting efficiency as with the full amount of reagents, we either prolonged the counting time in the gamma counter (Packard Instruments) to 2 min per sample or counted all samples to 10 000 cpm. For maximum sensitivity, we used the overnight procedure for all three RIA tests.

Samples
Serum and bone-marrow aspirates were obtained from patients in the departments of surgery (male controls), gynecology (female controls), hematology (bone-marrow aspirates from donors), and urology (patients with prostatic tumors) of our hospital. The male controls were selected men, ages 17 to 88 years, who were having hand or foot surgery. They were not rectally examined, but they had no signs of prostatic lesions. The bone-marrow aspirates were part of specimens from a few male and female patients with various malignant illnesses of the blood and lymph system or other cancers such as of the breast or bladder. Some male and female donor bone-marrow aspirates were obtained from bone-marrow transplantations done in this hospital.

Blood and bone-marrow aspirates were collected in Vacutainer Tubes (Becton Dickinson) without any additives and then divided into two portions: (a) heparinized containers for the enzymatic test, and (b) containers with no additives for the radioimmunological estimation of PAP. All samples were brought to the respective laboratories within 3 to 4 h after collection of the blood and either processed without delay (enzyme test) or stored frozen at −20 °C (RIA). For RIA the sera were allowed to come from −20 °C to room temperature without warming, then mixed by inversion and centrifuged. Lipemic sera were assayed but the lipemia was noted, lest it cause erroneous results. We used manual pipetting (Gilson pipettes) to distribute sera, Hamilton multiple syringes to dispense reagents. We assayed these samples by all three RIA tests concurrently.

Data Handling
Analysis of data. All data from the RIA tests were processed by a P-6060 Olivetti desktop computer. The RIA data were either calculated by using a four-parameter curve-fitting program (Amersham) or a logit-log program adapted to the P-6060. Using this computer and "statistics" programs from Olivetti, we statistically analyzed the data.

Evaluation of data. Data from day-to-day routine measurements were used in evaluating assay performance such as test sensitivity and precision.

We tested sera or bone-marrow samples for PAP without knowing details on the medical background of the patients. Only when all test results were available was the code broken and the data matched to the different groups, such as controls or stage of disease. Staging of prostatic cancer followed the “TNM-system” published by the Union Internationale Contra Cancer (UICC) (8).

The IDF method of Oehr et al. (5) was introduced for better representation of data: Enzyme concentrations were rank ordered sequentially according to decreasing antigen concentration. Every rank number was then divided by the rank number of the lowest concentration, and the result was multiplied by 100. Plotting the resulting values on the ordinate and the corresponding antigen concentration on the abscissa yielded the IDF plots shown later in Figures 3 and 6.

Clinical sensitivity (percent of true positives) and clinical specificity (percent of true negatives) at different thresholds could be obtained from the IDF curves by interpolating the percentages at these PAP concentrations (6).

Results

Analytical Variables

Standard curves: Figure 1 illustrates representative standard curves for the three RIA kits.

Total counts and nonspecific binding: The Mallinckrodt assay introduced the highest amount of radioactivity (60 000 cpm per tube), followed by NEN and Clinical Assays, both having 10 000 cpm per tube. Binding at zero dose ranged in all three kits from 30 to 60%, depending on the age of the kit. Nonspecific binding ranged from 3 to 8% in all three test systems.

Precision: Figure 2 shows the within-assay precision profiles.
for all three kits, based on the method recommended by Rodbard (9). Data from six standard curves, chosen without conscious bias, were subjected to this test. In the clinically relevant concentration range (cf. Table 2) the between-assay CVs were 6, 8, and 10% for Clinical Assays, NEN, and Mallinckrodt, respectively, corresponding to B/B₀ values in the standard curves between 70 and 75%.

Quality-control sera provided with the kits were included in the experiments and yielded PAP concentrations in the range indicated by the manufacturer, although the CV was rather large (Table 1); when we interchanged the quality-control sera provided with the three RIA kits, all three test systems yielded results that were in the correct range, but the individual results differed quite markedly (Table 1).

Clinical Data

'Serum: As measured with the four test systems, concentration ranges for PAP measured in control sera, sera of patients with benign hyperplasia of the prostate (BHP), and sera from women are summarized in Table 2. The PAP concentrations found in the individual sera of controls, patients with BHP, and untreated as well as treated carcinoma patients are plotted in Figure 3 in the form of IDF. As can be seen, there were striking differences between methods in the distribution of the individual PAP concentrations. Comparison of the inverse distribution of controls with BHP revealed that the four test systems discriminated among these groups differently.

Figure 3 gives the impression of an extremely low detection rate for prostatic carcinoma by the enzyme test, as compared with the three radioimmunoassays, but transformation of the data used in Figure 3 to compare the clinical performance (sensitivity and specificity) of the different test systems (Figure 4) shows that the enzyme test detected 35% of carcinomas of the prostate, and the radioimmunoassays between 45 and 60%. On the level of 35% true-positives, however, the enzyme test had a 15% chance of a falsely positive result (i.e., BHP mistakenly diagnosed instead of a carcinoma of the prostate). Among the radioimmunoassays, Clinical Assays has a slight advantage over the two other RIAs, because at a true-positive rate of 35%, it has only a 5% false-negative rate, as compared with 20% in the enzyme test.

PAP concentrations or activities as measured in untreated prostatic cancer patients are plotted in Figure 5 in relation to their clinical staging. Early stages of prostatic carcinomas exhibited mainly normal PAP concentrations, with no advantage of the RIAs over the enzyme test. This situation changed dramatically when the tumor reached stage T₃, where the tumor is thought to outgrow the boundaries of the prostatic gland; here the enzyme test was much less effective than the RIA. By stage T₄, the RIAs had no advantage over the enzyme test.

The three RIAs showed (Figure 3) lower PAP concentrations in treated than in untreated cancer patients.

Bone-marrow aspirates: We measured PAP in bone-marrow aspirates from male patients with no apparent prostatic tumor and from female patients who were undergoing an aspiration for diagnostic purposes or in preparation for bone-marrow transplantation, either as receivers or as donors (Table 3). Two patients with chronic myeloid leukemia and bone-marrow PAP activities of 4.9 and 6.4 U/L, respectively, were excluded. Greatly enhanced PAP concentrations and activities in bone marrow were detected in numerous patients.

---

### Table 1. Interchangeability of Quality-Control Sera

<table>
<thead>
<tr>
<th>Qual.-control sera</th>
<th>Clinical Assays</th>
<th>PAP, µg/L</th>
<th>Mallinckrodt</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1–2</td>
<td>11–12</td>
<td>2.3–5.3</td>
</tr>
<tr>
<td>CV, %</td>
<td>2.0 ± 0.3</td>
<td>11.5 ± 1.9</td>
<td>2.8 ± 0.6</td>
</tr>
<tr>
<td>NEN</td>
<td>2.7 ± 0.3</td>
<td>13.2 ± 1.4</td>
<td>3.3 ± 0.4</td>
</tr>
<tr>
<td>CV, %</td>
<td>2.0 ± 0.6</td>
<td>9.6 ± 2.2</td>
<td>10.0 ± 8.9</td>
</tr>
<tr>
<td>Mallinckrodt</td>
<td>3.0 ± 23</td>
<td>89</td>
<td>41</td>
</tr>
</tbody>
</table>

* Mean ± 1 SD (n = 10).

---

### Table 2. Reference Intervals

<table>
<thead>
<tr>
<th>Method</th>
<th>n</th>
<th>Sex</th>
<th>Range measured</th>
<th>Cutoff point</th>
<th>Range stated by manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzymic, a</td>
<td>216</td>
<td>δ</td>
<td>0.4–1.4</td>
<td>1.2 b</td>
<td>—</td>
</tr>
<tr>
<td>U/L</td>
<td>216</td>
<td>δ</td>
<td>0.4–1.9</td>
<td>1.9</td>
<td>—</td>
</tr>
<tr>
<td>Clinical Assays</td>
<td>172</td>
<td>δ</td>
<td>0.6–2.4</td>
<td>1.8 b</td>
<td>&lt;2</td>
</tr>
<tr>
<td>RIA, µg/L</td>
<td>19</td>
<td>δ</td>
<td>≤1.0–3.1</td>
<td>3.1</td>
<td>—</td>
</tr>
<tr>
<td>NEN RIA, µg/L</td>
<td>19</td>
<td>δ</td>
<td>0.4–1.3</td>
<td>1.2 b</td>
<td>—</td>
</tr>
<tr>
<td>Mallinckrodt</td>
<td>34</td>
<td>δ</td>
<td>0.3–2.5</td>
<td>2.3 b</td>
<td>—</td>
</tr>
<tr>
<td>RIA, µg/L</td>
<td>30</td>
<td>δ</td>
<td>≤1.0–6.5</td>
<td>3.0</td>
<td>0–6.5</td>
</tr>
<tr>
<td>RIA, µg/L</td>
<td>34</td>
<td>δ</td>
<td>0.5–1.8</td>
<td>1.7 b</td>
<td>0–1.6</td>
</tr>
</tbody>
</table>


---

Fig. 3, Inverse distribution function for PAP in 50 randomly selected control patients (V), patients with BHP (O), untreated (0) and treated (+) prostatic carcinoma

Some patients who exhibited PAP > 4 U/L (or 4 µg/L) were included in the calculations but not plotted.

2046 CLINICAL CHEMISTRY, Vol. 28, No. 10, 1982
with advanced prostatic cancer (data unpublished).

The IDF representation of the distribution of PAP concentrations or activities in bone marrow revealed a different picture from that observed with serum (Figure 3). The IDF curve was shifted to higher (1.5- to twofold greater) PAP concentration for bone marrow than for serum. Treated and untreated patients also showed a different distribution pattern for PAP in bone marrow as compared with serum patterns (Figure 6).

Analogous to our procedure with serum, we tried to evaluate the clinical effectiveness of PAP measurements in bone-marrow aspirates by comparing the curves obtained for PAP concentrations in bone-marrow aspirates of controls with PAP concentrations in untreated prostate carcinoma. On the level of a 10% chance of false-positives, the enzyme test detected 40% true-positives and the radioimmunoassays (NEN and Clinical Assays) 60 to 65% true-positives. The Mallinckrodt test yielded only data on the level of 20% false-positives. The detection of bone metastasis on this level is 70% for this test (Figure 7).

Table 4 compares the number of above-normal PAP values found for bone marrow of untreated and treated prostatic cancer patients. As this shows, more metastasis to the bone

**Table 3. Prostatic Acid Phosphatase in Bone Marrow**

<table>
<thead>
<tr>
<th>Test</th>
<th>n</th>
<th>Range</th>
<th>$\bar{x} \pm 1$ SD</th>
<th>Upper limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzymic test, U/L</td>
<td>51</td>
<td>0–12.7</td>
<td>1.3 ± 0.5</td>
<td>2.7</td>
</tr>
<tr>
<td>Clinical Assays RIA, µg/L</td>
<td>49</td>
<td>≤1.0–17.7</td>
<td>1.4 ± 0.6</td>
<td>3.0</td>
</tr>
<tr>
<td>Mallinckrodt RIA, µg/L</td>
<td>49</td>
<td>≤1.0–16.4</td>
<td>1.7 ± 0.6</td>
<td>3.8</td>
</tr>
<tr>
<td>NEN RIA, µg/L</td>
<td>49</td>
<td>≤1.0–32.6</td>
<td>1.7 ± 0.5</td>
<td>3.0</td>
</tr>
</tbody>
</table>
Table 4. PAP in Bone Marrow of Untreated and Treated Prostatic-Cancer Patients above the Respective Limits (cf. Table 3) Compared with Data Obtained by Scintigraphy

<table>
<thead>
<tr>
<th>Stage</th>
<th>Enzyme</th>
<th>Clinical Assays</th>
<th>Mallinkrodt</th>
<th>NEN</th>
<th>Scintigraphy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T0–2</td>
<td>0 (3)</td>
<td>1 (5)</td>
<td>2 (5)</td>
<td>2 (5)</td>
<td>1 (6) (17%)</td>
</tr>
<tr>
<td>T3</td>
<td>1 (9)</td>
<td>4 (9)</td>
<td>4 (9)</td>
<td>3 (6)</td>
<td>2 (16) (13%)</td>
</tr>
<tr>
<td>T4</td>
<td>4 (8)</td>
<td>7 (8)</td>
<td>7 (8)</td>
<td>8 (8)</td>
<td>9 (12) (75%)</td>
</tr>
<tr>
<td>Total</td>
<td>5 (20)</td>
<td>12 (22)</td>
<td>13 (22)</td>
<td>13 (19)</td>
<td></td>
</tr>
<tr>
<td>Treated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T0–2</td>
<td>0 (1)</td>
<td>0 (1)</td>
<td>0 (1)</td>
<td>0 (1)</td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>2 (8)</td>
<td>4 (8)</td>
<td>3 (6)</td>
<td>3 (6)</td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>3 (4)</td>
<td>4 (4)</td>
<td>4 (4)</td>
<td>4 (4)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>5 (13)</td>
<td>8 (13)</td>
<td>7 (11)</td>
<td>7 (11)</td>
<td></td>
</tr>
<tr>
<td>Overall detected (n)</td>
<td>10 (33)</td>
<td>20 (35)</td>
<td>20 (33)</td>
<td>20 (30)</td>
<td>12 (34)</td>
</tr>
<tr>
<td></td>
<td>30%</td>
<td>57%</td>
<td>60%</td>
<td>67%</td>
<td>35%</td>
</tr>
</tbody>
</table>

a Listing contains both treated and untreated patients. b No. in parentheses indicates total no. of cases examined.

were detected by radioimmunological measurement of PAP than by bone-scan scintigraphy.

Discussion

PAP in Serum

If a well-known and inexpensive method of established merit is to be replaced by a more expensive and more laborious method, success rates in detecting illness must be much higher with the new test. Introducing the immunological principle in the measurement of PAP raised expectations that PAP could in the future be used as a tumor-characteristic antigen as is alpha-fetoprotein or chorionicadotropin in tumors of the testis. The immunological principle showed promise of circumventing the problems with the enzyme test such as the enzyme's sensitivity to pH and heat and nonspecific reactions with acid phosphatases from other sources. We compared the three radioimmunooasays with the thymolphthalein monophosphate-based enzyme test to establish whether they lived up to the expectations for a tumor-marker test.

Differentiation between malignant and benign lesions by biochemical methods requires high precision in the clinically relevant concentration range. As seen in Figure 2, the three RIAs tested show acceptable CVs.

With the availability of so many radioimmunological test systems the clinician often is confronted with PAP estimates from these different assays. It would therefore be desirable to intercompare test results. Experiments in this direction on interchanging quality control sera together with simultaneous assays of serum samples show the impossibility of such an attempt (Table 1). The introduction of an international standard would improve the situation, but would not entirely solve the problem, because antisera used in kits potentially differ in specificity from test to test.

In the course of dilution studies (data not shown) we used a pooled specimen of serum with zero PAP concentration (from women) as the diluent in the Clinical Assays and NEN kits. However, this serum pool consistently gave values of 3 μg/L in the Mallinkrodt test. On the basis of these results and the warning given by Mallinkrodt to use only the material provided with the kit, we conclude that severe matrix effects can be the reason for erroneously high values for PAP. Our preliminary data on dilution studies with use of bone-marrow aspirates confirm this.

On the other hand, on measuring PAP in serum or bone-marrow aspirates by either enzymic or immunological methods, one encounters the situation of possible endogenous sources of prostatic acid phosphatases or of an acid phosphatase-like substance, with properties closely similar to the enzyme produced by prostatic tissue or by metastatic cells in bone marrow. The two patients with chronic myeloid leukemia in this study did not exhibit any signs of a prostatic lesion, but their sera showed above-normal PAP concentrations and their bone-marrow aspirates showed even higher concentrations. Yam et al. (10) recently described a tartrate-sensitive isoenzyme—no. 2 by polyacrylamide electrophoresis—from a patient with neutrophilic leukemia, which reacted with antiserum to PAP isolated from human seminal fluid. They claim that normal subjects also exhibit this prostatic acid phosphatase-like isoenzyme activity. On the grounds of our findings in the two patients with chronic myelogenous leukemia and the report by Yam et al., we conclude that neutrophils can contribute to falsely positive PAP values.

The RIA for PAP has been propagated because of its claimed insensitivity to molecular changes in PAP on exposure to heat or on prolonged standing in unclotted blood. The reports by Josephson and Houle (11) and Geller and Albert (12), which show that the immunological activity of PAP also can be diminished on prolonged standing of the sera, prompted us to look at data available in our laboratory on repeatedly frozen and thawed sera. We made the disquieting observation that freezing and thawing, previously claimed by Foti et al. (13) to be of no importance, can have an effect on the immunologically detectable PAP in some serum samples (Table 5). This phenomenon is not restricted to a special antisera, as shown by Josephson and Houle (11), who demonstrated the same effect with antisera of other origins.

Clinical Relevance of PAP Measurements

The information obtained from the measurement of antigen
in a control population is usually restricted to establishing the "normal range" or the "cutoff value" for the antigen. Moreover, it is very difficult to compare on an equal basis the performance of the four tests examined. Introducing the "inverse distribution function" provided information on the respective specificity and sensitivity of the PAP tests (5, 6).

The IDF allows researchers to set the antigen concentration not just to one specially defined value, but to the value of antigen that they think fits best with their clinical experience. Figure 3 on serum PAP and especially Figures 4 and 5 show that none of the tests examined is an ideal marker for prostatic cancer.

Because in all four tests quite a large proportion of patients with BHP showed measurable PAP concentrations and many prostatic-cancer patients showed low concentrations, PAP would best be used as prostatic marker, rather than a marker specific for prostatic cancer. Figure 5 makes it quite evident that PAP cannot be used as an early tumor marker but can still be useful in establishing that the prostatic carcinoma has broken through the capsule of the gland. Because the treated carcinomas showed lower PAP concentrations than did their untreated counterparts (Figure 3), we believe that PAP assay can also be used to monitor therapy of prostatic tumors (14).

Prostatic Acid Phosphatase in Bone Marrow

The finding of Chua et al. (15) that PAP was not only increased in sera of patients with advanced stages of prostatic carcinoma but possibly in aspirates of bone marrow as well increased hopes for a clearer and earlier recognition of metastasis of prostatic carcinoma. However, the composition of the bone marrow and its connection to the blood system brings up some questions. The bone marrow is rich in cells of the erythropoietic system, which contain high concentrations of acid phosphatase of nonprostatic origin. Therefore, measurements of PAP by enzyme techniques in bone marrow is beset with problems (15-17). Therefore, the RIA, with its presumably higher specificity, was introduced by several workers (18-20). Led by the optimistic views coming from their work in recognizing bone metastasis at relatively early stages of the disease, and because knowledge of bone metastasis can determine the route of therapy, we applied the four available techniques to measure PAP in the bone-marrow aspirates available to us and compared the results with those obtained by bone scan and (or) roentgenogram. The bone marrow was obtained from the spina iliaca sup., but according to Belville et al. (21), PAP concentration does not depend on the location from which bone marrow is sampled.

PAP concentrations from bone-marrow aspirates, measured with both the enzymic and the immunological method, were consistently higher than the corresponding concentrations in serum. Such a finding was made earlier by Bruce et al. (22). We were tempted to explain these discrepancies by disruption of bone-marrow cells upon aspiration of the bone marrow, with consequent release of large amounts of acid phosphatases cross reacting in the tests. The specificity of the RIAs did not allow such an explanation, nor would the curves in Figure 6 run parallel for serum and bone marrow if this were so, but would have an erratic distribution pattern. We prefer to explain this phenomenon by a subtle difference in the composition of serum and bone marrow, i.e., a matrix effect.

Patients with prostatic metastasis to the bone in several cases showed much greater PAP concentrations in bone marrow than in serum, even more than could be explained by the matrix effect alone. Testing such aspirates in dilution experiments with the RIAs gave no definite answer to the question of the identity of PAP-like activity in bone marrow with that in serum, because consistent dilution curves could not be obtained in all patients tested (data not shown). We concluded that bone marrow, especially from patients with advanced prostatic cancer (i.e., with extended bone metastasis) and from patients with some forms of leukemia, contains not only PAP from metastatic cells of the prostate but also a protein having some PAP activity derived from cells of the bone marrow that have been activated by the process of metastasis. This substance presumably is only moderately released to the serum compartment, which would account for the accumulated apparent PAP activity in bone marrow in comparison with serum.

We thank the Scientific Foundation of the Kantonsspital Basel, Basel, Switzerland, for financial help, and the companies Byk-Mallinckrodt (F.R.G.) and Travensol (Clinical Assays, Boston, MA), for providing some of the test material used in this study.

References