Acid and Alkaline Glycerophosphatase in Tissue and Serum*

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(Received for publication February 21, 1942)

Since the publication by Kay (9) of a method for the determination of the glycerophosphatase of blood serum, increasing interest has been manifested in the occurrence of phosphatases in the blood and in various organs in health and disease. It was early established that the phosphatase of bone has its maximum activity in alkaline solution (17), and that of the prostate gland is active only in acid solution (10, 11). Some confusion existed for a time regarding the pH of maximum activity of the phosphatases of various other tissues. The reason for this became apparent when several independent investigators showed that the liver and kidney contain both acid and alkaline phosphatases which can be separated (1). The Gutmans and their coworkers have shown clearly that bones which are the site of metastases from carcinoma of the prostate contain large amounts of acid phenylphosphatase and alkaline glycerophosphatase (4-8). They have also established the clinical significance of the occurrence of these enzymes in the blood. The phosphatase of bone is active only in acid solution (10, 11).

Substrate is present in large excess at all times. The method employed for tissue phosphatase was based originally on that of Franseen (3) but has been modified so that the results may be more directly comparable with those for serum. One to 5 gm. portions of the fresh tissue are minced finely with scissors or morcelled with rongeurs and weighed (into a flask). Twenty cc. of water per gm. of tissue are then added together with a few drops of toluene, and the mixture is kept in the icebox with occasional shaking for 48 to 72 hours. It is then filtered through washed gauze, and the filtrate used for phosphatase determination.

A few modifications of the method for serum phosphatase are necessary in handling tissue extracts owing to the wide range of phosphatase activities and inorganic phosphorus concentrations encountered in the latter. Usually a trial run is necessary before the best conditions can be found. For inorganic phosphate determinations a mixture of 3 cc. of filtrate with 12 cc. of 5 per cent trichloracetic acid often gives a convenient phosphate concentration, but proportions of 0.5:14.5 or 7.5:7.5 must sometimes be used. In the last case 10 per cent trichloracetic acid instead of 5 per cent must be used so as to avoid too low a final acidity. As the phosphatase activities to be measured range from 0.05 units to 500 units per gm. of tissue, the times of incubation must be varied inversely. Incubation times from 2.5 minutes to 24 hours may be used. The relation of time of incubation to amount of phosphate liberated is discussed in detail below.

We have introduced several modifications in the method as originally developed (2). In a previous publication (14) we described a procedure for correcting for reagent blanks. This renders the method somewhat more flexible and assures that contamin-

METHOD

The method employed for alkaline serum phosphatase is essentially that of Bodansky (2). This consists in brief in adding 1 volume of serum to 9 volumes of a water solution containing 0.5 per cent sodium β-glycerophosphate and 0.42 per cent monosodium diethylbarbiturate. The mixture is incubated for 1 hour at 37° C., the enzyme is then inactivated by the addition of an equal volume of 10 per cent trichloracetic acid, and the mixture is filtered. At the same time 1 volume of serum is added to 9 volumes of 5 per cent trichloracetic acid in another container, and filtered. The inorganic phosphate in the 2 filtrates is then determined by adding to each 5 cc. of filtrate 4 cc. of a solution of sodium molybdate in sulfuric acid, followed by 1 cc. of a solution of stannous chloride in hydrochloric acid, and comparing with a standard in a colorimeter. The amounts of phosphorus in the filtrates are calculated to those which would have been obtained from 100 cc. of serum under like conditions, and the amount in the unincubated specimen is subtracted from that in the incubated specimen. The difference represents the amount of substrate decomposed by phosphatase. One unit of phosphatase is defined as a phosphatase activity which will liberate 1 mg. of phosphorus as phosphate ion from the given substrate during 1 hour incubation at 37° C. under the experimental conditions described. Substrate is present in large excess at all times.

Our method for tissue phosphatase was based originally on that of Franseen (3) but has been modified so that the results may be more directly comparable with those for serum. One to 5 gm. portions of the fresh tissue are minced finely with scissors or morcelled with rongeurs and weighed (into a flask). Twenty cc. of water per gm. of tissue are then added together with a few drops of toluene, and the mixture is kept in the icebox with occasional shaking for 48 to 72 hours. It is then filtered through washed gauze and the filtrate used for phosphatase determination.

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* This investigation was aided by a grant from The Anna Fuller Fund.
tion of reagents or deterioration of substrate will be discovered. We have found, however, that the correction curves as originally published are not of general validity, but must be redetermined for each lot of reagents. With this technic, satisfactory readings may be obtained when the concentration of the unknown is between one-third and twice the concentration of the standard. When the phosphatase activity is such that the concentration of the unknown is more than twice that of the standard after 1 hour incubation, a shorter incubation is desirable. Readings not more than 15 per cent in error may usually be obtained on unknowns with as high as 5 times the concentration of reagents or deterioration of substrate will be discovered. We have found, however, that the correction curves as originally published are not of general validity, but must be redetermined for each lot of reagents. With this technic, satisfactory readings may be obtained when the concentration of the unknown is between one-third and twice the concentration of the standard. When the phosphatase activity is such that the concentration of the unknown is more than twice that of the standard after 1 hour incubation, a shorter incubation is desirable. Readings not more than 15 per cent in error may usually be obtained on unknowns with as high as 5 times the concentration of the standard, however, by taking only 1 or 2 cc. of filtrate and making up to 5 cc. with water. In this case, since the phosphatase correction factor represents the inhibitory effect of the substrate on color development, and since smaller quantities of substrate are present, only one-fifth or two-fifths of the usual correction is made. When the concentration of the unknown is more than 5 times that of the standard, the determination indicates order of magnitude only and must be repeated with appropriate reduction in incubation time.

Some standard makes of filter paper contain significant amounts of phosphate and must not be used. We have always found Whatman No. 1 to be free of contamination and to retain precipitate satisfactorily. Turbid filtrates are occasionally encountered with blood from jaundiced patients or with certain tissue extracts, but these can be cleared by passing a second time through the same filter paper.

Adjustment of pH

Bodansky's substrate, when mixed with serum in the proportions of 10:1, gave a colorimetric pH of 8.6. We early encountered difficulty in preventing absorption of carbon dioxide by the substrate with consequent decrease in pH. We therefore adopted the practice of withdrawing 1 cc. from each serum-substrate or tissue extract-substrate mixture for pH determination. Originally readings for alkaline phosphatase were made in the bicolorimeter with thymol blue; readings for acid phosphatase were made in the comparator with propyl red or bromothymol blue. For most of the work to be reported in the present paper, pH determinations were made with the Beckman pH meter. Fair checks were obtained between readings in the acid range made by the two methods. For alkaline serum-substrate mixtures, however, a serious protein error was found to be present in the colorimetric pH readings, a colorimetric reading of 8.6 being equivalent to an electrometric reading of 9.1. We have, therefore, adopted 9.10 as the pH of reference for alkaline phosphatase determinations.

In practice, alkaline serum phosphatases are usually determined at approximately pH 9.0 and 9.2, and the value at pH 9.1 found by interpolation. Alkaline phosphatase of tissue must always be determined in this way because, as will be explained later, the pH curves of different tissue phosphatases differ enormously. The phosphatase pH curve for serum between pH 8.9 and 9.4, on the other hand, is nearly constant over a wide range of phosphatase activities. For this reason, when it is inconvenient to make duplicate determinations of alkaline serum phosphatase, or when, owing to shift in the substrate alkalinity or to unusually high or low protein content of the serum, the pH values of both serum-substrate mixtures lie on the same side of pH 9.1, the phosphatase readings at the experimental pH may be calculated to those at pH 9.1 by means of the correction curve in Fig. 1. In this curve the phosphatase activity at pH 9.10 is assumed to be unity. The pH values at which the determinations are made are plotted as abscissas against the factors necessary to correct the activities to pH 9.10 as ordinates. The curve was constructed from the results of 165 determinations made on 51 sera whose phosphatase activities ranged from 1.4 to 38.5 units per 100 cc. The deviations of the different points from the curve were not greater than the maximum error of the colorimetric readings. About 500 additional duplicate serum phosphatase readings have been corrected to pH 9.10 by means of the curve with satisfactory results. Inspection of the curve shows that, in the neighborhood of pH 9.10, a shift of 0.10 pH causes a change of about 14 per cent in serum phosphatase activity. The necessity of careful pH control is at once apparent.

Phosphatase determinations in the pH range between 8.0 and 10.5 are made in the usual buffered substrate to which appropriate small amounts of hydrochloric acid or sodium hydroxide have been added. Phosphatase determinations at a pH below 8.0 are made in a solution containing 0.5 per cent sodium glycerophosphate and no buffer. The pH is adjusted to the desired value by addition of hydrochloric acid. The buffering actions of different tissue extracts differ considerably. The buffering action of serum is also somewhat variable, although averaging higher than that of tissue extracts. Hence, the exact amount of acid or alkali which must be added to any given mixture of tissue extract or serum with substrate must be determined by trial. A rough guide to final acid and alkali concentrations which may be expected to give certain pH readings to mixtures of 9 parts of
Unbuffered substrate

<table>
<thead>
<tr>
<th>pH</th>
<th>0.013 HCl</th>
<th>0.0125 &quot;</th>
<th>0.0115 &quot;</th>
<th>0.0080 &quot;</th>
<th>0.0030 &quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>

Buffered substrate

<table>
<thead>
<tr>
<th>pH</th>
<th>0.015 HCl</th>
<th>0.0 &quot;</th>
<th>0.025 NaOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.5</td>
<td>9.0</td>
<td>10.0</td>
<td></td>
</tr>
</tbody>
</table>

Spontaneous acid hydrolysis of the substrate is negligible under the experimental conditions. Samples of unbuffered substrate which had been brought to pH values of 2.3 to 6.4 and incubated for 45 hours at 37°C. failed to show free phosphate ion in concentrations which could be demonstrated with certainty by the colorimetric method. Unbuffered substrate containing a drop of toluene keeps for a month or more in the icebox at its natural pH of about 8.4. At pH values of 6.0 or less it readily becomes moldy with rapid liberation of phosphate ion. Hence, we do not add acid to the stock supply of substrate even when numerous determinations are to be run at the same pH, but adjust the pH just before the solution is to be used.

When solutions containing phosphatase are mixed with substrate and incubated, the pH of the mixture may change. When the incubation is for 1 hour or less, the change is usually small. In a previous publication (13) we stated that, in alkaline serum phosphatase determinations, the average change in the colorimetric pH of the serum substrate mixture during incubation was −0.09 pH. Recently, using the pH meter, we have found the average in 21 alkaline phosphatase determinations to be only −0.01 pH or less than the experimental error. We conclude that the changes previously observed in colorimetric pH may have been due to changes in the color of the solutions. For determinations on acid serum phosphatase incubated 1 to 4 hours, there is often a slight rise in pH, but as the phosphatase activity is nearly constant over a considerable range of acidity, this may be disregarded. In tissue phosphatase determinations where long incubations are necessary, the change in pH may be significant. Thus, for 20 alkaline tissue phosphatases incubated 17 to 23 hours, the average change was −0.12 pH and the range was −0.39 pH to +0.06 pH. For 33 acid tissue phosphatases the average change was −0.05 pH and the range was −0.32 pH to +0.23 pH. With long periods of incubation it is therefore desirable to read the pH at both the beginning and the end of the experiment and use the average.

**TIME OF INCUBATION**

Bodansky (2) published correction factors to be applied when times of incubation longer or shorter than 1 hour are employed. We have found that the concentration of phosphate ion liberated from substrate by serum phosphatase action is nearly always directly proportional to time of incubation. This is illustrated in Fig. 2A. Forty-eight determinations were made on 16 sera whose phosphatase activity varied from 2.8 to 155.5 units. Times of incubation ranged from 2.5 minutes to 3 hours. In each experiment the phosphatase reading which had the phosphate concentration nearest to that of the standard was arbitrarily assigned a value of unity, and its time of incubation was termed standard time ($T_s$). The ratios of other times of incubation ($T_i$) to standard time were then found and plotted against the corresponding ratios for the amount of phosphate liberated by phosphatase ($P_{ase}$). The reason for employing this time of incubation instead of 1 hour as the standard is that, with very high phosphatase values, incubation for 1 hour results in the liberation of phosphate in concentrations too high to be read in the colorimeter. In the figure, experimental values are represented by crosses and the theoretical ratios by the solid line. It is seen that only 3 of the points deviate from the theoretical line by more than the experimental error of the colorimetric method when the phosphate concentration of the filtrate is between one-fourth and 3 times that of the standard.

For tissue phosphatase the proportionality between time of incubation and concentration of phosphate liberated holds only when the final phosphate concentration is between one-half and three-halves that of the standard. This is illustrated in Fig. 2B, which comprises 19 determinations of acid and alkaline phosphatase on 6 tissue extracts. The phosphatase values ranged from 1.7 to 15.5 units per gm., and the times of incubation from 2.5 minutes to 2 hours.

This behavior of tissue phosphatase is similar to that reported (2) for serum. It is not apparent why, for the majority of sera, we fail to confirm Bodansky's findings, nor why, in our hands, serum phosphatase and tissue phosphatase behave differently. It is possible that under some conditions the products of hydrolysis tend to inhibit phosphatase activity, and that these conditions are realized regularly in tissue extracts but only rarely in serum. The mechanism of inhibition is unknown and deserves further study.

It is impossible to determine experimentally what the readings on extracts containing 0.10 units per gm. or 500.0 units per gm. would be after incubating...
1 hour. In practice, therefore, we incubate both tissue extracts and sera for whatever times will give convenient final phosphate concentrations. We then calculate the number of milligrams of phosphorus liberated per gram of tissue during the incubation period employed and multiply the result by the quotient of 60 divided by the number of minutes of incubation. Thus our phosphatase unit, while reduced to the value at 60 minutes for convenience, is based primarily on the time required to liberate a given amount of phosphate, rather than on the amount of phosphate liberated in given time.

Fig. 1.—pH correction curve for serum alkaline phosphatase. Abscissas, pH at which determinations are made. Ordinates, factors for correcting activities at experimental pH to activities at pH 9.10.

Fig. 2.—Relation of concentration of phosphate liberated to time of incubation. $P_{\text{ase}}/P_{\text{ase},s} =$ ratio of concentration at experimental time to concentration at standard time. $T_e/T_s =$ ratio of experimental time to standard time.

Fig. 3 and 4.—Tissue phosphatase activities in units per gram plotted against pH at which determination is made.

Fig. 3.—Normal bone.
A. Cortex of normal growing radius.
B. Cortex of normal adult femur.
C. Cancellous portion of young adult tibia.

Fig. 4.—Pathological bone. Acid phosphatase plotted on scales 10 and 100 times that of alkaline phosphatase.
A. Curettings from os calcis showing bone rarefaction and fibrosis.
B. Osteogenic sarcoma, femur.

Dilution

In general, as explained above, we employ a dilution of 9 volumes of substrate to 1 volume of serum or tissue extract. With very high phosphatase activities, such as are sometimes encountered in the serum of patients with Paget's disease, or in extracts of...
prostatic or osteogenic sarcoma tissue, it is impossible to obtain satisfactory readings without further dilution. We have tested the effect of dilution on a few osteogenic sarcoma extracts, the time of incubation being varied inversely with the concentration, so that all final phosphate concentrations would be of the same order of magnitude. Results indicated that there is about a 20 per cent decrease in the phosphatase activity per gram of original tissue with a tenfold increase in dilution, but that a twofold change in dilution causes no significant change in activity. The effect of dilution on different preparations is not necessarily the same, since it is probable that phosphatase activity is influenced by coenzyme systems whose nature is not known at present, but whose activities may be affected by dilution in a different manner from that of phosphatase itself. It is recommended, therefore, that dilutions of phosphatase preparations be changed as little as is compatible with the use of accurately measurable times of incubation.

**Effect of Standing**

It has been shown by Bodansky (2) and confirmed by us (13) that the alkaline phosphatase activity of serum increases significantly on standing. Hence, we always determine serum phosphatase as soon as possible after the blood is drawn, and never preserve the specimens more than 18 hours. In contrast to serum phosphatase, the phosphatase activity of tissue extracts changes little on standing in the icebox for periods up to 2 months, provided no precipitate forms. This is illustrated in the following figures:

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Time of standing, days</th>
<th>Alkaline phosphatase, units per gm.</th>
<th>Acid phosphatase, units per gm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osteogenic sarcoma</td>
<td>1</td>
<td>22.0</td>
<td>1.17</td>
</tr>
<tr>
<td>&quot;</td>
<td>15</td>
<td>18.8</td>
<td>1.07</td>
</tr>
<tr>
<td>&quot;</td>
<td>39</td>
<td>19.2</td>
<td>1.04</td>
</tr>
<tr>
<td>&quot;</td>
<td>59</td>
<td>22.0</td>
<td>1.03</td>
</tr>
<tr>
<td>&quot;</td>
<td>79</td>
<td>20.1</td>
<td>1.02</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>5</td>
<td>1.22</td>
<td>1.04</td>
</tr>
<tr>
<td>&quot;</td>
<td>13</td>
<td>1.67</td>
<td>1.04</td>
</tr>
<tr>
<td>&quot;</td>
<td>23</td>
<td>1.58</td>
<td>1.04</td>
</tr>
<tr>
<td>&quot;</td>
<td>40</td>
<td>1.45</td>
<td>1.04</td>
</tr>
</tbody>
</table>

Many extracts of soft-part tissues flocculate after a week or 10 days. The phosphatase adsorbs on the precipitate and, as this usually cannot be redispersed evenly by shaking, attempts at determining the activity of such extracts lead to erratic results. Highly turbid extracts which seem likely to flocculate should, therefore, be studied within 3 or 4 days of preparation. Examination of clear extracts, especially those of bone, may, on the other hand, be deferred for 1 or 2 weeks when desired.

**RESULTS**

**Bone Phosphatase**

When phosphatase activities are determined at different acidities and alkalinities and the activities are plotted against the pH values at which they were made, curves are obtained which are to some extent characteristic of the tissue of origin. Particularly is this true of bone phosphatase. This is illustrated in Fig. 3 by the phosphatase-pH curves for extracts of normal growing bone and of adult bone; and in Fig. 4 by similar curves for fibrotic hyperplastic bone and osteogenic sarcoma. It is seen that there is a sharp maximum in the alkaline range. The exact position of this maximum is hard to determine accurately, owing to the difficulty of securing exactly the desired pH, but it is nearly always close to pH 9.40. This is very near to the pH of maximum activity of the alkaline phosphatase of serum as shown in Fig. 1, and lends further weight to the evidence of numerous workers that the major portion of the alkaline phosphatase of serum is of osseous origin.

For the sake of uniformity, it was felt to be desirable to retain the same pH of reference (pH 9.10 electrometric or pH 8.6 colorimetric) for alkaline tissue phosphatase as that established by Bodansky (2) for serum. It is fortunate that this pH is not that of the maximum activity of bone phosphatase, since it would be too time-consuming to locate this point accurately as a routine procedure. It is comparatively easy, however, by determining phosphatase at approximately pH 9.00 and 9.20, to find the value at pH 9.10 by interpolation. It must be borne in mind, however, that the values for alkaline phosphatase reported in this paper are in general lower than the maximum obtainable.

While it is obvious that the bone extracts illustrated in Figs. 3A and 4 had an enormously greater phosphatase activity in alkaline solutions than in neutral or acid solutions, yet such extracts nearly always contain a small amount of acid phosphatase. In Fig. 4 the acid phosphatase values are plotted both on the same scale as the alkaline and on a scale 10 or 100 times as large. Inspection of the large-scale plot shows that the alkaline phosphatase is active down to pH 6.0, and that in still more acid solutions a true acid phosphatase makes its appearance. The presence of this acid phosphatase is more easily demonstrated in extracts of normal bone in which the alkaline phosphatase activity is low. This is illustrated in Fig. 3B and 3C by the phosphatase-pH curves for extracts of shaft of adult femur and epiphysis of young adult...
The alkaline phosphatase activities are low, especially in the femur, in which the activity is only about 1/500 that of the osteogenic sarcoma shown in Fig. 4B, and the maxima are less sharp than in the more active extracts. The presence of an acid phosphatase with a broad maximum between pH 4.0 and 5.0 is easily seen. This acid phosphatase probably originates in nonosseous elements in bone or in interstitial fluid. The values for acid phosphatase reported in this paper were all determined at the pH of maximum activity.

**Normal Bone**

A sufficient number of specimens of healthy human bone have been examined to permit the establishment of normal values for alkaline phosphatase. These are summarized below. As no significant differences were found among the various long bones, values for femur, tibia, fibula, humerus, ulna, radius, and clavicle are grouped together.

<table>
<thead>
<tr>
<th>Bone of specimens</th>
<th>Number of specimens</th>
<th>Alkaline phosphatase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult long bone, cortex</td>
<td>12</td>
<td>Average, units per gm.</td>
</tr>
<tr>
<td>&quot; &quot; &quot; cancellous portion</td>
<td></td>
<td>Range, units per gm.</td>
</tr>
<tr>
<td>Children’s long bone, cortex</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>&quot; &quot; &quot; cancellous portion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adult rib cortex</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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</table>

Acid phosphatase activities were studied on 15 of the 36 specimens. Barely detectable amounts (0.01 to 0.03 units per gm.) were found in the cortex of long bone, whereas cancellous bone and rib contained 0.10 to 0.40 units per gm. This acid phosphatase probably originated in the marrow, small portions of which were unavoidably included in the bone selected for extraction.

The wide range of values encountered in children’s bones undoubtedly reflects variations in the growth rate. In the cortex of adult long bone the alkaline phosphatase activity is exceedingly low, while in the adult rib it is 4 to 10 times as high. As the function of phosphatase in nongrowing bone is presumably to provide phosphate for the repair of ordinary wear and tear, we may infer that the continual bending strains to which ribs are subjected makes necessary a much more active repair mechanism than that of long bone. In adult long bone we feel that an alkaline phosphatase activity of 0.5 units per gm. or more indicates that the bone has recently been subjected to trauma or that some pathological process is at work.

A few determinations on flat bones and vertebrae indicate that their phosphatase activities are higher than those of other parts of the skeleton. Sufficient data are not yet at hand for the establishment of normal values for these bones.

The greatly increased alkaline phosphatase activity manifested in bone which is the site of an attempt at repair is illustrated in Fig. 4A. Five specimens of hyperplastic bone have been examined. In 3 the diagnosis was "fibrosing osteitis," in 1 "bone rarefaction and fibrosis," and in 1 "osteomyelitis." The alkaline phosphatase ranged from 1.8 to 28.5 units per gm., and the acid phosphatase from 0.10 to 0.28 units per gm.

**Bone Tumors**

Tissue phosphatase activities have been determined on 33 specimens of osteogenic sarcoma from 25 patients. None of these tumors had received preoperative irradiation. The alkaline phosphatase readings ranged from 0 to 115.0 units per gm. Acid phosphatase readings were made on 18 of the specimens and ranged from 0.02 to 0.80 units per gm. In the majority of cases the phosphatase-pH curves were definitely of the bone type shown in Fig. 4. This was true not only of most of the primary tumors but also of the 3 specimens of metastases to lung. There were, however, all gradations between this type of curve and one closely resembling that found for the giant cell tumors and endothelioma of bone to be discussed later. In several a wide difference in the phosphatase activities of different portions of the same tumor was found. Dr. F. W. Stewart of this Hospital has kindly reviewed the slides on many of these specimens and has been unable to find any correlation between histological type and phosphatase activity. We conclude, therefore, that the alkaline phosphatase activity of osteogenic sarcoma tissue reflects the functional state of the tumor at the time of examination regardless of the tissue elements which predominate. The possible implications of this observation have been discussed in more detail elsewhere (16).

Four specimens of osteochondroma were examined. The values for acid and alkaline phosphatase found were only slightly above those which would have been
expected in the normal bones from which these benign, slow-growing tumors arose.

Alkaline phosphatase determinations were made on 10 specimens of benign and malignant giant cell tumors. The alkaline phosphatase values ranged from 0.10 to 2.3 units per gm, but were above 0.50 units in only 4 specimens. All of these were atypical or frankly malignant. The onset of malignant tendencies is not necessarily associated with a rise in the alkaline phosphatase. Acid phosphatase determinations were made on only 4 of the specimens of giant cell tumor. In these the acid phosphatase values were found to range from 0.52 to 1.9 units per gm. While further work is necessary for definite conclusions, it seems probable that giant cell tumor tissue itself contains principally acid phosphatase. Where considerable amounts of alkaline phosphatase are found, this probably originates in the adjacent intact bone and is a manifestation of an attempt at repair such as takes place when the bones are invaded by metastases from tumors of soft-part origin.

We have had very little opportunity to examine active tissue from endothelioma of bone because this tumor is nearly always treated by roentgen irradiation before being removed surgically. We have, however, obtained 4 postmortem specimens of metastases and one untreated primary tumor. The acid phosphatase values of these were from 0.30 to 1.5 units per gm. The only specimen containing more than 0.15 units per gm. of alkaline phosphatase was a metastasis to rib with considerable new bone formation. The curve for this tissue, together with that for a soft-part metastasis from the same patient, appears in Fig. 5. The 2 curves are quite different. This is in contrast to the behavior of osteogenic sarcoma in which the metastases, even when not calcified, usually have phosphatase-pH curves indistinguishable from that of the primary. The situation in endothelioma of bone, which arises from endothelial rather than osseous elements, is probably analogous to that just discussed for giant cell tumor. Osteogenic sarcoma, on the other hand, arises from osseous tissue, and this origin is reflected in the capacity of most of the primary tumors and their metastases to manufacture alkaline phosphatase.

**Muscle**

In extracts of 6 specimens of normal skeletal muscle and 1 specimen of normal uterine muscle no alkaline phosphatase activity was found. Four of these specimens had barely detectable activities in the acid range (less than 0.05 units per gm.). Evidently the β-glycerophosphatases measured by the method employed here are not concerned in the mechanism for carbohydrate metabolism in muscle. Two specimens of uncalcified uterine fibroid behaved like normal muscle. The findings on 5 specimens of abnormal muscle and tumor arising from muscle are of interest and are summarized below.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Acid phosphatase, units per gm.</th>
<th>Alkaline phosphatase, units per gm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atrophic fibrotic muscle</td>
<td>0.0</td>
<td>0.21</td>
</tr>
<tr>
<td>Edematous muscle</td>
<td>0.06</td>
<td>0.28</td>
</tr>
<tr>
<td>Spindle cell sarcoma probably of muscle type; calcification in tumor, not true ossification</td>
<td>0.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Same tumor, soft necrotic area</td>
<td>0.15</td>
<td>0.95</td>
</tr>
<tr>
<td>Myositis ossificans showing dead bone in tendon or aponeurosis</td>
<td>0.10</td>
<td>8.7</td>
</tr>
<tr>
<td>Adjacent muscle, normal in the gross</td>
<td>0.03</td>
<td>0.03</td>
</tr>
</tbody>
</table>

The first 2 specimens had undergone mild pathological changes such as sometimes lead to calcification, and contained small but significant amounts of alkaline phosphatase. The third and fourth specimens were of a malignant tumor arising in, and probably from, muscle. One was calcified and the other not, but both contained abundant alkaline phosphatase. In the fifth specimen, which was from an area of myositis ossificans in an 8-year-old boy, the muscle had been entirely converted into bone. This abnormal bone had a higher alkaline phosphatase activity than we have observed in any normal bone, even in growing children. Only questionable alkaline phosphatase activity was found in the adjacent uninvolved muscle of the same patient. Wilkins, Regen, and Carpenter (12) have reported the presence of high alkaline glycerophosphatase activities, not only in calcified myositis ossificans tissue but also in muscle in the pre-ossification stage. These findings suggest that a derangement of the phosphorylation mechanism of muscle leading to the appearance of alkaline glycerophosphatase precedes calcification in ossifying muscle lesions, and may be the primary cause of these disturbances. Further studies on such tissues will be made as they become available.

**Liver and Kidney**

As stated in the introduction, it is known that liver and kidney contain both acid and alkaline phosphatase. Phosphatase-pH curves for specimens of these 2 tissues are given in Fig. 6. We have only a small series of observations on human liver and kidney, but have encountered considerable variation in both the relative and absolute amounts of acid and alkaline phosphatase, with consequent variations in the shapes of the curves. While the tissues were normal in the...
gross, they were obtained from patients who had died of cancer, and the profound nutritional disturbances which preceded death may well have altered the metabolism of the liver and kidney. Hence, we can make no statement as to the phosphatase of these organs in normal man. We have, however, begun, in collaboration with Dr. K. Sugiura of this Hospital, an investigation of the livers of rats on a diet containing butter yellow. Preliminary observations indicate that the onset of malignant changes is associated with definite increase in phosphatase activity, especially in the alkaline range.

Prostate

While the presence of an enormous acid phosphatase activity in extracts of human prostate gland and of prostatic carcinoma is too well known to require detailed consideration here, it is of considerable theoretical and practical interest to determine the exact pH range over which this phosphatase is active. In Fig. 7A is presented the phosphatase-pH curve for an extract of a prostate showing benign hypertrophy and chronic interstitial prostatitis. In Fig. 7B is shown a similar curve for a fibromyomatous prostate. The left-hand portion of one curve and the right-hand portion of the other are also shown on a larger scale. There are small differences between the 2 curves, but in both there is a broad maximum between pH 4.0 and 5.5. At pH values lower or higher than this, the activity decreases rapidly. It is small below pH 2.0 and above pH 8.0 and disappears entirely above pH 8.5.

Testes

The alkaline phosphatase of 4 testes normal in the gross was found to range from 0 to 0.11 units per gm., and the acid phosphatase from 0.23 to 1.3 units per gm. As only one of the specimens was from a young man, the relation of phosphatase to functional activity is not known. In contrast to these findings on normal testis, the alkaline phosphatase reading on a primary embryonal adenocarcinoma of the testis was 1.3 units per gm., and the readings on 2 metastatic deposits from a similar tumor in another patient were 19.5 and 27.5 units per gm. The acid phosphatase of the 3 specimens ranged from 0.65 to 1.4 units per gm., or not much higher than in normal testes. The discovery in testicular tumors of an alkaline phosphatase activity higher than that which has been observed to date in any other tissue except regenerating bone and osteogenic sarcoma is entirely unexpected and will be studied further.

Carcinoma and Lymphomatoid Tissue

We have examined 7 specimens of carcinoma tissue and 6 of lymphomatoid tissue. In all, the phosphatase-pH curves were of the double type found for liver and kidney. The acid phosphatase figures ranged from 0.23 to 1.6 units per gm. The alkaline phosphatase activities averaged considerably lower and exceeded 0.40 units per gm. in only 2 cases. One of these was an adenoid cystic adenocarcinoma of salivary gland origin with 1.7 units per gm.; the other was lymphomatoid tissue from a patient with Hodgkin's disease and had an alkaline phosphatase activity of 0.68 units per gm.

The curves for 2 specimens of myeloid myeloma tissue, one invading muscle and one invading bone, appear in Fig. 8. The patient was a man of 50 years. The disease apparently originated in the marrow of the clavicle and extended through the cortex of the bone and into the soft parts. The soft mass, which was free of bone and muscle, contained 0.70 units per gm. of acid phosphatase and only 0.15 units per gm. of alkaline phosphatase. The mixed tissue consisted of a spongy framework of regenerating bone enclosing myeloma. The acid phosphatase activity of the extract of the mixture was about the same as that of the pure myeloma. It is evident that this came from the myeloma and not from the bone, since the activity of extracts of normal clavicle in the acid range is extremely low. This finding is analogous to the demonstration (8) of the presence of prostatic phosphatase activity in metastases to bone from carcinoma of the prostate, the only difference being in the very much smaller acid phosphatase activity of the myeloma tissue. The alkaline phosphatase activity of our bone specimen amounted to 3.6 units per gm. When it is remembered that normal adult clavicle does not contain more than 0.50 units per gm. of alkaline phosphatase, and usually much less, it is evident that the presence of a tumor tending to destroy bone resulted in a great increase in the activity of the repair mechanism of the osseous tissue. We have made similar observations on tissue from metastatic areas in bone from carcinoma of the breast. The curves in Fig. 8 are also similar to those in Fig. 5 for endothelioma of bone. It is, therefore, a frequent finding that bones which are the site of metastases from tumors of soft-part origin produce alkaline phosphatase in amounts far in excess of those produced in similar normal bones. This constitutes a direct proof of the theory which we postulated (15) from the indirect evidence obtained by the study of phosphatase in the serum of patients with metastatic bone disease.
SERUM

The phosphatase-pH curves for the serum of a normal young woman and of a normal young man appear in Fig. 9A and 9B, respectively. We have

![Fig. 9A](image)

![Fig. 9B](image)

5.0 and a minimum at about pH 6.5. Absolute values in the acid range were low. For a series of 12 females and 13 males in whom the alkaline phosphatase did not exceed 5.0 units per 100 cc., the average acid

![Fig. 5](image)

![Fig. 6](image)

obtained similar curves from numerous normal individuals and have not observed significant differences between males and females. Besides the well-known maximum of activity at about pH 9.5 there was in all curves a broad maximum between pH 4.0 and phosphatase determined between pH 4.0 and 5.0 was 0.28 units per 100 cc., with a range of 0.04 to 0.64 units per 100 cc. The group included 9 normal persons and 16 patients with miscellaneous diseases not involving the prostate gland. There was no significant
difference in the average and range of readings for males and females. It is evident that human serum contains small amounts of acid glycerophosphatase which are not derived from the prostate gland. This is in harmony with the findings of the Gutmans (5) for phenylphosphatase.

It will be remembered from Fig. 7 that the activity of extracts of prostatic tissue at pH 6.5 is only 20 to 30 per cent less than that at pH 4.5, while the activity of bone phosphatase at pH 6.5 is commonly less than 10 per cent of that at pH 9.1. The activity of the mixture of phosphatases in normal human serum is at a minimum at pH 6.5, although this activity is only about 30 per cent less than that at the acid maximum. When the minimum of activity of normal serum at pH 6.5 was first discovered, it was thought that this was the point at which the presence of prostatic phosphatase could be demonstrated with the least confusion from alkaline phosphatase or nonprostatic acid phosphatase. We employed this pH of reference for about 200 determinations of acid serum phosphatase and found the results useful in diagnosing metastatic carcinoma of the prostate. The method was not entirely satisfactory because, in patients with very high alkaline serum phosphatase readings, the readings at pH 6.5 were not clear-cut owing to residual alkaline phosphatase activity at this pH. In the attempt to eliminate this difficulty we have now adopted pH 4.5 as our reference point for acid serum phosphatase.

Even at pH 4.5 we have not been completely successful in avoiding the effects of alkaline phosphatase. In patients without prostatic cancer, in whom the possible to exclude residual alkaline phosphatase activity entirely except at a pH below that of maximum activity for acid phosphatase. In practice we find it most satisfactory to determine the acid serum phosphatase at pH 4.5 and make allowance for possible effects of alkaline phosphatase in interpreting the results.

In order to demonstrate that the behavior of prostatic phosphatase with respect to the pH of the substrate is the same in serum as it is in extracts of prostatic tissue, we have prepared the curve for serum phosphatase in Fig. 10B. The patient was a man of 52 with a moderate degree of bone involvement and very extensive soft-part metastases from carcinoma of the prostate. It is seen that the left-hand position of the curve resembles very closely those for prostatic extracts in Fig. 7. The right hand portions are of
course different, as the serum contains considerable alkaline phosphatase originating in the regenerating areas of the involved bones.

The clinical significance of our determinations of acid serum phosphatase will not be discussed in detail here. In summary, we believe that the presence of a serum phosphatase activity at pH 4.5 of 0.7 to 1.0 units per 100 cc., when the alkaline phosphatase is normal, warrants the suspicion that the patient has metastasizing carcinoma of the prostate; and we feel that this diagnosis is very probable when the acid phosphatase exceeds 1.0 units per 100 cc. If the alkaline serum phosphatase is elevated, then a phosphatase activity at pH 4.5 of 1.5 units per 100 cc. or more appears to be pathognomonic of metastasizing carcinoma of the prostate. In doubtful cases the acid phosphatase may be determined at both pH 4.5 and pH 6.5. If the reading at pH 4.5 is higher than that at pH 6.5, then there is probably a true elevation of acid phosphatase rather than a high residual activity of alkaline phosphatase in the acid range. If the acid serum phosphatase is elevated and the alkaline phosphatase is normal, the patient probably has carcinoma of the prostate metastatic to soft parts but not to bone. If both acid and alkaline phosphatases are elevated, there are metastases to bone from carcinoma of the prostate. If both phosphatases are elevated and the acid is higher than the alkaline, it is likely that there are prostatic metastases to both bone and soft parts. We have not seen significant elevations in the acid serum phosphatase of patients with carcinoma of the prostate without metastases. On the other hand, the presence of a normal acid serum phosphatase gives no assurance that metastases from carcinoma of the prostate are not present.

SUMMARY

A method is described for measuring the action of serum and of crude tissue extracts on sodium-β-glycerophosphate over the pH range from 3.0 to 10.0.

The alkaline glycerophosphatase activity of the cortex of normal adult human long bone has been found to range from 0.04 to 0.15 units per gm. Corresponding values for children's long bones are 0.16 to 3.3 units per gm. Acid phosphatase activities in cortical bone were barely detectible.

Regenerating bone has been found to contain up to 50 times as much alkaline glycerophosphatase as normal bone.

A range of alkaline glycerophosphatase activity from 0 to 15.0 units per gm. has been found in extracts of osteogenic sarcoma tissue. No correlation has been found between phosphatase activity and histological type. Soft-part metastases from osteogenic sarcoma have alkaline glycerophosphatase activities of the same order of magnitude as the primary tumor.

The glycerophosphatases of extracts of giant cell tumors and of endothelioma of bone are active mainly in acid solution. Significant alkaline glycerophosphatase activities are found only when portions of regenerating bone are included in the specimen.

Areas of bone invaded by metastases from tumors of soft part origin contain more alkaline glycerophosphatase than does normal bone.

Acid and alkaline glycerophosphatase activities of the order of 0.5 to 1.5 units per gm. have been found in extracts of normal liver and kidney, and in most specimens of carcinoma and lymphomatoid tissue.

No alkaline glycerophosphatase and very small amounts of acid glycerophosphatase have been found in extracts of normal muscle. Significant amounts of alkaline glycerophosphatase have been found in a few extracts of pathological muscle.

Some evidence has been obtained that the alkaline glycerophosphatase of embryonal adenocarcinoma of the testis is much higher than that of normal testis.

Prostatic glycerophosphatase has been found to be active from pH 2.0 to 8.0. Slight activity was occasionally detected at a pH as high as 8.3, but none in solutions more alkaline than this.

The significance of acid phosphatase readings on the serum of patients with carcinoma of the prostate is discussed.

The author wishes to express her appreciation to members of the Pathological Department under Dr. F. W. Stewart, the Bone Department under Dr. B. L. Coley, and the Urological Department under Dr. Benjamin Barringer for their cooperation in furnishing specimens of tissue and for assistance in interpreting results. The author is also indebted to Miss Maria Chianti and Miss Mildred Moore of the Chemical Department for technical assistance.

REFERENCES


Acid and Alkaline Glycerophosphatase in Tissue and Serum

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