

# Colorimetric determination of serum acid phosphatase activity using adenosine 3'-monophosphate as substrate

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**SYNOPSIS** The hydrolysis of adenosine 3'-monophosphate by serum acid phosphatase has been coupled to the liberation of ammonia from the adenosine generated through the action of exogenous adenosine deaminase. The ammonia is measured at the end of the incubation by a modification of the phenol-hypochlorite reaction of Berthelot. Optimum conditions for the enzyme reaction have been defined. Inhibition of the Berthelot reaction by the serum used in the assay is small, and may be compensated by a correction factor. Although the value for the control is high in relation to the test over the normal range, this is largely outweighed by the good sensitivity and precision of the method.

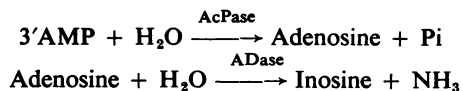
The substrate is not significantly hydrolysed by erythrocyte acid phosphatase within the limits encountered in haemolysed sera. Experience of the method in routine hospital diagnosis compared favourably with that of a standard method employing disodium phenyl phosphate as substrate. It is suggested that activities greater than 3.1 IU/l should be further investigated and those greater than 3.7 IU/l should be regarded as definitely raised. The stability of human serum AcPase when promptly separated and held at 4°C or -20°C was confirmed. At room temperature, acidification to pH 6.0 greatly improved stability.

The determination of acid phosphatase (AcPase, EC 3.1.3.2, orthophosphoric monoester phosphohydrolase) activity of human serum has been established as a laboratory procedure in the diagnosis of prostatic cancer (Gutman and Gutman, 1938; Woodard, 1959), metastatic mammary cancer (Joplin and Jegatheesan, 1962; Jegatheesan and Joplin, 1962), and Gaucher's disease (Ockerman and Kohlin, 1969).

Many substrates have been used in this determination. These include adenosine 3'-monophosphate (3'AMP) (Fischman, Chamberlin, Cubiles, and Schmidt, 1948; Reynolds, Reynolds, and Walker, 1956). This has the convenient property of being poorly hydrolysed by erythrocyte AcPase (Tsuboi and Hudson, 1953) and yet provides an excellent substrate for prostatic AcPase (Tsuboi and Hudson, 1955). Its failure to gain wider acceptance relates to the fact that hydrolysis has in the past been measured by the rather tedious techniques of protein precipitation and phosphate estimation.

Recently, we have shown that hydrolysis of

3'AMP can be coupled through adenosine deaminase (ADase, EC 3.5.4.4, adenosine aminohydrolase) to produce inosine and ammonia under conditions optimal for AcPase (Belfield and Goldberg, 1970). The following reactions are involved:



The decrease in extinction at 265 nm consequent upon conversion of adenosine to inosine may be measured spectrophotometrically and provides a method that is rapid and sensitive employing tissue preparations with moderate AcPase activity, but cannot cope with the high absorbance of serum at the wavelength used. An alternative possibility lay in determining ammonia production utilizing the phenol-hypochlorite reaction of Berthelot as modified by Chaney and Marbach (1962). This procedure is described in the present paper, which also presents some preliminary clinical data comparing the proposed method with a standard technique in routine hospital diagnosis.

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## Materials and Methods

### CLINICAL CATEGORIES

Blood samples were obtained from three groups of subjects.

#### Group A

This comprised 36 healthy members of the laboratory staff aged 18-40 years.

#### Group B

This comprised 97 subjects over the age of 65 attending a special general practitioner screening clinic. All were ambulant; a few were being treated for chronic non-malignant disease and were well compensated at the time of examination.

#### Group C

This comprised samples of venous blood drawn from 137 subjects referred for laboratory examination because of prostatic symptoms or possible metastatic bone involvement.

### REAGENTS

All were of highest analytical grade and all water used was glass distilled and de-ionized.

1 *0.1 M Acetate buffer*  
pH 5.6 at 20°C.

#### 2 Adenosine deaminase

Obtained from Boehringer Corporation (London) Ltd as a suspension in 50% (v/v) glycerol (catalogue no. 15069 EAAT).

#### 3 Acetate buffer/ADase

One  $\mu$ l of neat ADase suspension was added per ml of buffer, the mixture being prepared fresh daily.

#### 4 3'AMP (80 mM)

NaOH is required to dissolve the free acid, and the final pH must lie between 5 and 8. This was stored at -20°C in aliquots of about 2 ml, a fresh aliquot being used each day.

#### 5 3'AMP/ADase

One  $\mu$ l of neat ADase was added per ml of substrate, the mixture being prepared fresh daily.

#### 6 Phenol/nitroprusside

#### 7 Hypochlorite

The above two reagents were in the concentrated form described by Chaney and Marbach (1962).

#### 8 Ammonium standard (2 mM)

### TECHNIQUE

For each serum, a test and control were run, and with each batch, standards and reagent blanks were put through. The protocol is set out in Table I.

	Test	Control	Standard	Reagent Blank
Acetate buffer/ADase (ml)	0.9	0.9	0.9	0.9
Ammonia standard ( $\mu$ )	—	—	50	—
Water ( $\mu$ l)	—	—	50	100
Serum ( $\mu$ l)	50	50	—	—
<i>Equilibrate 5 minutes at 37°C</i>				
3'AMP/ADase ( $\mu$ l)	50	—	—	—
<i>Incubate exactly 1 hour at 37°C</i>				
Phenol/nitroprusside (ml)	1	1	1	1
3'AMP/ADase ( $\mu$ l)	—	50	—	—
Hypochlorite (ml)	1	1	1	1
<i>Incubate at 37°C for 30 minutes and read extinction at 630 nm against water</i>				

Table I Protocol for determination of serum AcPase activity using 3'AMP as substrate

Batches were processed in such a way that the test and control for each serum were incubated for an identical period before adding the phenol/nitroprusside reagent.

Activity as IU/l was given by the expression

$$\frac{E_{\text{test}} - E_{\text{control}}}{E_{\text{standard}} - E_{\text{reagent blank}}} \times 33.3 \times 1.04$$

The derivation of the factor 1.04 which corrects for depression of the Berthelot reaction by serum is described later.

## Results

### DEVELOPMENT OF THE METHOD

#### Choice of buffer

A pH activity curve was constructed over the range 4.5-6.5 using 0.1 M acetate and 0.1 M citrate buffers. The pH was measured at 37°C before and after incubation, using the Micro-Astrup apparatus (Radiometer Corporation, Copenhagen, Denmark). Optimal activity was obtained at pH 5.6 with both buffers (Fig. 1). Comparison of a number of buffers adjusted to 0.1 M and pH 5.6 at 37°C yielded the following activities relative to acetate: citrate 0.95, succinate/borate 0.84, phosphate 0.20, citrate/phosphate 0.16, veronal/acetate 0.68, phthalate 0.81. The buffers were prepared from standard tables (Geigy, 1956). Varying molarity of acetate from 0.05 to 0.9 M did not affect activity. Since ammonia generated by a very active serum produced negligible change in pH of 0.1 M acetate buffer even when

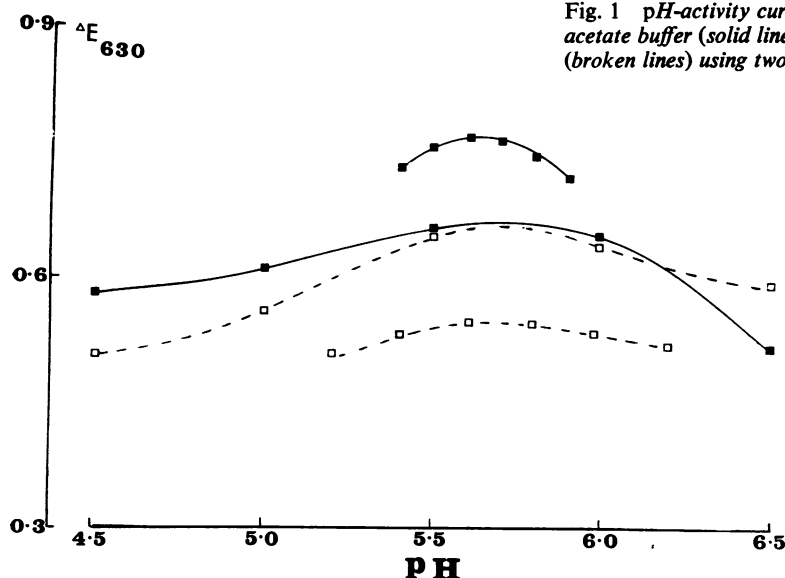


Fig. 1 pH-activity curves for serum AcPase in 0.1 M acetate buffer (solid lines) and 0.1 M citrate buffer (broken lines) using two sera of high activity.

the incubation was extended to six hours, this strength was deemed appropriate. The addition of NaCl in final concentrations of 0.08 to 0.32 M inhibited AcPase activity; this was 80% of the control at 0.32 M.

#### Choice of substrate

The criteria governing this choice were that the substrate should have a high affinity towards prostatic AcPase and low affinity towards erythrocyte AcPase. Of the monophosphates of adenosine tested, 3'AMP best fulfilled these criteria (Table II).

Substrate (4 mM)	Activity as $\Delta E_{630}$ (Test - Control)	
	Prostate <sup>1</sup>	Erythrocytes <sup>2</sup>
2'AMP	0.096	0
3'AMP	0.294	0
5'AMP	0.107	0.229

Table II Relative AcPase activities of human prostate and erythrocytes using various monophosphates of adenosine as substrate

<sup>1</sup>50  $\mu$ l of a 1 : 200 (v/v) mixture of human prostatic AcPase in pooled human serum.

<sup>2</sup>50  $\mu$ l of a 1 : 10 water haemolysate of washed packed human erythrocytes.

In this study purified human prostatic AcPase prepared according to the method of Belfield and Goldberg (1970) was dialysed until free of ammonium sulphate and 1 part added to 200 parts of pooled human serum. The  $K_m$  for 3'AMP was

determined utilizing a serum of high AcPase activity and an incubation time of 10 minutes and 11 concentrations of substrate in the range 0.046-1.824 mM final concentration. Statistical evaluation of the data using a computer program (Hoy and Goldberg, 1971) gave a value of 0.391 with standard error  $\pm 0.055$ . The concentration in the definitive assay, being 10 times  $K_m$ , sustains 91% of theoretical  $V_{max}$ .

#### Adenosine deaminase

The preparation used was free of AcPase activity. Using a serum of high AcPase activity, it was found that a final concentration of 0.1  $\mu$ l/ml of the neat ADase preparation supported the maximal reaction rate over 60 minutes. This is one-ninth of the ADase concentration of the definite assay.

#### Required blanks

The recommended method allows for the ammonia originally present in the serum as well as that generated from sources other than AMP during the incubation, since the serum is incubated under conditions identical with those of the test but omitting substrate. It is also necessary to take account of ammonia and adenosine present as a contaminant of AMP. Further allowance is made for generation of adenosine from AMP during incubation at 37°C. All of the above sources of ammonia, other than that due to specific hydrolysis of AMP by AcPase of serum, were fully allowed for in the control employed in the definitive assay.

*Characteristics of colour reaction*

The reaction gave values for  $\Delta E_{630}$  linear with ammonia concentration to an absorbance of 2.60. At 37°C, and with the reagents of the definitive assay, absorbance reached a maximum within 10 minutes of adding hypochlorite, and remained stable for a further 10 minutes, thereafter decreasing at a rate of 0.05% per minute at room temperature. No loss of ammonia took place from stoppered tubes under the assay conditions even when incubation at 37°C was continued for 18 hours.

The presence of protein during development of the Berthelot reaction reduced the colour yield from added ammonia. This inhibition increased non-linearly with increasing amount of serum; although reduced in the presence of serum, the absorbance was always linear with ammonia added to values of 2.50. This enabled the use of a correction factor to allow for this inhibition. Using 50  $\mu$ l serum and the conditions of the enzyme assay, the percentage recovery of ammonia obtained with 24 sera gave a mean  $\pm$  SD of  $95.9 \pm 3.1$ , hence the factor of 1.04 used in calculation of enzyme activity. This may be employed only for determinations at pH 5.6 with 50  $\mu$ l serum.

When the colour was developed at 56°C, not only was the yield reduced, but the colour became progressively unstable as the amount of serum was increased. Other studies to overcome this inhibition were unsuccessful. They were similar to experiments described in an earlier report (Belfield, Ellis, and Goldberg, 1970).

*Characteristics of enzyme reaction*

The definitive procedure yielded activity proportional to the amount of enzyme up to  $\Delta E_{630}$  of 1.10, corresponding to approximately 58 IU/litre. The reaction, even with sera of very high activity, was linear with time up to at least one hour. It is recommended that assays should be repeated using a shorter incubation time where  $\Delta E_{630}$  after one hour exceeds 1.10; this maintains the validity of the correction factor used in calculating activity.

The within-batch precision of the method gauged by 13 replicate assays of a serum gave a mean of 50.1 IU/l with the coefficient of variation = 2.4%. The SD of pooled duplicates was derived according to Pearson (1942) from analyses on the 137 patients in group C. The range and median value of this case material is given in Table IV and the SD of the duplicates was 0.8 IU/litre.

## EFFECT OF HAEMOLYSIS

Heparinized and clotted blood were obtained from a normal individual. The latter was centrifuged to obtain the serum and the former to obtain the

erythrocytes which were washed twice with ice-cold isotonic saline, packed by centrifugation, and ruptured by freezing and thawing. The haemolysate was added in varying amounts to the serum, and the AcPase activity of the mixture measured by the present technique as well as by the method of Gutman and Gutman (1940) with and without formaldehyde in a final concentration of 0.5%. Although the total AcPase as determined by the latter method was greatly increased by addition of haemolysate, no significant change in activity by the present technique or in the formal-stable AcPase took place up to a haemolysate concentration of 2% (Table III).

Haemolysate Concentration as Percentage of Serum Volume	3'AMP (IU/l)	Substrate for AcPase Activity	
		Phenyl Phosphate <sup>1</sup> (Total)	Phenyl Phosphate <sup>1</sup> (Formol-stable)
0	1.4	2	2
0.10	0.9	2	1
0.67	1.2	5	1
2.00	1.8	10	1

Table III Effect of haemolysis on AcPase activity using 3'AMP and disodium phenyl phosphate as substrates

<sup>1</sup>Determined by method of Gutman and Gutman (1940) as units per 100 ml.

## STABILITY OF HUMAN SERUM ACPASE

This was tested by drawing a large sample of venous blood from a patient with high activity due to advanced prostatic cancer. The serum was separated without delay and split into three major aliquots, one being untreated, a second being adjusted to a final concentration of 0.1% with respect to Triton X-100, and a third adjusted to a pH of approximately 6.0 by adding 10  $\mu$ l of 20% (v/v) acetic acid per millilitre serum. All three lost approximately 10% activity due to the initial freezing, but thereafter no significant change took place at -20°C over a period of six months when fractions stored individually to avoid the need for repeated freezing and thawing were assayed. Neither was there any significant change in the three preparations when stored at 4°C for up to eight days; but at room temperature the activity of the untreated and detergent-treated preparations declined steadily, whereas the activity of the acid-treated preparation was unchanged (Fig. 2).

## OBSERVATIONS ON HUMAN SUBJECTS

The activities observed in the three groups of subjects studied are summarized in Table IV. Because the distribution was skewed in all groups, the data were plotted on probability paper to give the 95-percentile range. Where, as occasionally happened, the value for the control exceeded that of

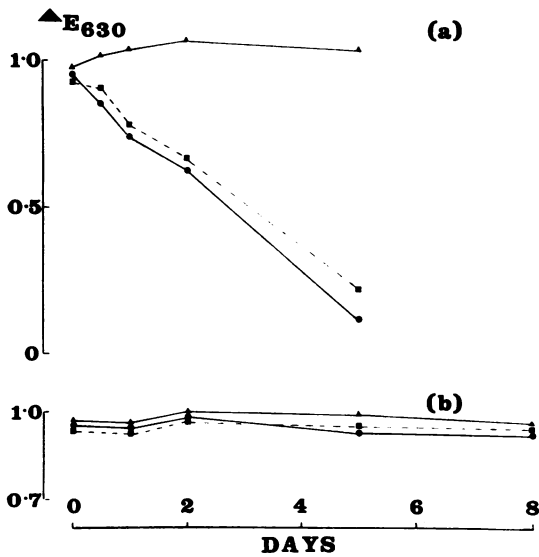


Fig. 2 Stability of serum AcPase activity at room temperature (a, top) and at 4°C (b, bottom) when untreated (circles) and when treated with acid (triangles) or detergent (squares).

the test, a negative activity was assigned to the sample so that the ranges take full account of the experimental error.

The upper limit for the laboratory staff (group A) was lower than that of the elderly population (group B) which in turn was lower than the hospital population (group C), but the median values for all three were similar. The total AcPase activity of all patients in group C was also determined by the method of Gutman and Gutman (1940). The upper normal limit for this laboratory is 3 units per 100 ml. Where this value was exceeded, the assay was repeated at the earliest opportunity in the presence of a final concentration of 0.5% formaldehyde, a value in excess of 2 units per 100 ml being regarded as abnormal. A statistical analysis of the AcPase activities, as measured by the two methods in the same patients, yielded a correlation coefficient of 0.248 ( $P < 0.01$ ) and the regression equation,

$y = 1.00 + 0.31x$ , where  $y$  and  $x$  are AcPase activities in Gutman units and IU/l respectively. The standard errors of the slopes and intercept were 0.11 and 0.14 respectively. Clearly, the two methods are not reflecting an identical enzyme activity, values for the Gutman procedure being relatively greater at low AcPase levels, presumably because that method includes a contribution from erythrocyte AcPase.

An analysis of those cases in group C where a value of 3.1 IU/l was exceeded appears in Table V.

Case No.	Clinical Details	Serum AcPase Activity	
		Gutman and Gutman (1940) <sup>a</sup>	Present Method (IU/l)
1	Prostatic cancer stage II	2	3.5
2	Prostatic cancer stage II	2	3.7
3	Prostatic cancer stage III	3 (1)	4.9
4	Prostatic cancer stage III	5 (2)	6.9
5	Benign prostatic hypertrophy	2	4.1
6 <sup>1</sup>	Benign prostatic hypertrophy	2	3.3
7 <sup>2</sup>	Benign prostatic hypertrophy	4 (2)	5.5
8 <sup>1,3</sup>	Benign prostatic hypertrophy	2	7.5
9 <sup>1</sup>	Benign prostatic hypertrophy	4 (0)	3.5
10 <sup>1</sup>	Benign prostatic hypertrophy	1	3.9
11 <sup>3</sup>	Benign prostatic hypertrophy	2	6.5
12	Benign prostatic hypertrophy	2	3.3
13	Non-prostatic bone secondaries	1	6.0
14	Non-prostatic bone secondaries	5 (1)	7.9
15	Non-prostatic bone secondaries	5 (2)	5.7
16	Severe burns, bone biopsy	2	4.1
17 <sup>2</sup>	Urinary tract infection	2	4.2
18 <sup>3</sup>	Diverticulitis, spinal opacity	3 (1)	7.2
19 <sup>3</sup>	Renal stone	3 (1)	3.4
20	Notes unavailable	1	3.4

Table V Details of patients from group C with serum AcPase activity > 3.1 IU/l by present method

<sup>1</sup>No operation or biopsy

<sup>2</sup>Catheter *in situ* at time of sample

<sup>3</sup>Sample taken within two hours of rectal examination

<sup>a</sup>Units per 100 ml, formol-stable activity in parenthesis.

Cases 1-4 had prostatic cancer which was intra-capsular (stage II) in two and had spread to soft tissues (stage III) in two; total AcPase measured by the Gutman procedure was raised in only one of these cases, and none had elevated formol-stable AcPase. Cases 5-12 had benign prostatic hypertrophy, but surgical and pathological confirmation was available in only three of these eight cases; total AcPase by the Gutman procedure was raised in two

Group	Number of Subjects			95-Percentile Range of Serum AcPase (IU/l)		
	Total	Males	Females	Lower Limit	Median	Upper Limit
A	36	19	17	0.9	1.8	3.1
B	97	41	56	0.7	2.1	3.7
C	137	133	4	-0.4	1.2	5.1

Table IV Serum AcPase values in three groups of human subjects using 3' AMP as substrate

of these cases and formol-stable AcPase in none. Cases 13-15 had non-prostatic malignant bone secondaries; total AcPase by the Gutman procedure was raised in two and formol-stable AcPase in none. Cases 16-20 had miscellaneous conditions itemized in Table V, and total AcPase by the Gutman procedure was normal in all. Seven of the patients in Table V in categories other than prostatic or bone cancer were catheterized at the time of venepuncture, or had blood taken within two hours of rectal examination.

Five cases, presented in Table VI, had normal serum AcPase with 3'AMP as substrate but raised activity as determined by the Gutman procedure, although in only one case was the formol-stable activity abnormal (4 units). One was a case of stage II prostatic cancer; noteworthy is the fact that formol-stable AcPase in this case was normal, the sample being grossly haemolysed.

Case No.	Clinical Details	Serum AcPase Activity	
		Gutman and Gutman (1940) <sup>a</sup>	Present Method (IU/l)
21	Prostatic cancer stage II	12 (1)	0.2
22	Benign prostatic hypertrophy	6 (4)	2.4
23	Non-prostatic bone secondaries	5 (2)	1.1
24	Diabetes, cervical spondylosis	4 (2)	0.8
25 <sup>b</sup>	Acute postoperative retention	4 (1)	1.4

Table VI Details of patients from group C with serum AcPase activity > 3 Gutman units per 100 ml but < 3.1 IU/l by present method

<sup>a</sup>Catheter *in situ* at time of sample.

<sup>b</sup>Units per 100 ml, formol-stable activity in parentheses

Current experience thus suggests that serum AcPase activity  $\leq 3.1$  IU/l by the present technique is normal, between 3.2 and 3.7 IU/l possibly abnormal, and > 3.7 IU/l definitely abnormal. These criteria should permit detection of a high proportion of cases of prostatic cancer, although the false-positive rate is also likely to be high unless precautions are taken to avoid trauma to the prostate at the time of sampling. This problem may be accentuated by the higher specificity of 3'AMP for prostatic AcPase.

## Discussion

### CRITIQUE OF METHOD

The method presented is convenient and simple to perform. Although the colour given by the control is high in relation to that of the test in the normal range, the precision with which ammonia in both test and control can be measured is high and the sensitivity of the Berthelot reaction very great, so

that the measured extinction difference covers an adequate span in the normal range. The inclusion of a correction factor for inhibition of the Berthelot reaction by serum is designed to permit strict comparison on a molar basis between 3'AMP hydrolysis and that of other substrates. For purely clinical use it can probably be ignored, as it is small and consistent. The colour is read at 30 minutes, by which time it has declined 0.5% from its peak value. This happens to be a matter of convenience in our laboratory, but the error in selecting any time between 15 and 40 minutes would be negligible.

### SUBSTRATE SPECIFICITY

Since the work of Abul-Fadl and King (1949), which described the differential effects of various inhibitors, including formaldehyde and L-tartrate, upon AcPase activity of several organs and cells, it has been common to describe tartrate-labile AcPase as 'prostatic'. This identity was never claimed by the original authors, and much subsequent work has shown the occurrence of tartrate-labile AcPase in other tissues, notably the liver (Nelson, 1966; Brightwell and Tappel, 1968). A similar misconception has arisen regarding the use of  $\alpha$ -naphthyl phosphate as substrate. Although claimed to be virtually specific for prostatic AcPase (Babson and Read, 1959; Babson and Phillips, 1966), it has now been shown not to be so (Amador, Price, and Marshall, 1969), and indeed many years earlier it was reported to be hydrolysed by an enzyme in female urine (Altschule, Parkhurst, and Zager, 1951). Erythrocyte AcPase has a very low affinity for  $\beta$ -glycerophosphate (Tsuboi and Hudson, 1953), but the virtual identity of the range of values using this substrate found for serum AcPase activity of normal men and women (Woodard, 1959) precludes its specificity for prostatic AcPase. The unequivocal demonstration of prostatic AcPase in human serum would seem at present to rest with polyacrylamide gel electrophoresis (Rozenszajn, Epstein, Shoham, and Arber, 1968).

Nevertheless, two of the substrates used in existing methods, as well as 3'AMP, have in common the desirable feature of being poorly hydrolysed by erythrocyte AcPase, and it would seem appropriate that routine diagnostic determinations should be based on one of those three. The reagents used in the  $\alpha$ -naphthyl phosphate method are more costly and less stable than those of the present method, and four times as much serum is needed, except where the sensitive spectrofluorimetric assay of Campbell and Moss (1961) is used. Hydrolysis of  $\beta$ -glycerophosphate is determined by assay of the phosphorus liberated. Such assays are associated with relatively high values for the control; sensitivity is not as great as when ammonia is assayed (Belfield, Ellis, and

Goldberg, 1970); the methods, when used in a routine laboratory, give unsatisfactory precision (Belfield and Goldberg, 1969); and contamination is a continual problem (Tsuboi and Hudson, 1953; Belfield and Goldberg, 1971). Apart from its technical difficulties, determination of tartrate-labile AcPase appears to offer no advantage over total AcPase in the diagnosis of prostatic cancer (Murphy, Reynoso, Kenny, and Gaeta, 1969) and the case for continuing its use is difficult to sustain.

#### STABILITY OF SERUM ACPASE

This study was undertaken in view of the reported instability of prostatic AcPase (Tsuboi and Hudson, 1955; Kilsheimer and Axelrod, 1957) and serum AcPase (Jacobson, 1960); the first two sets of authors used Triton X-100 to stabilize the enzyme against surface inactivation while the latter author acidified the serum to prevent pH-dependent inactivation. It was thought that this instability might be the cause of frequent failure to confirm high values by the techniques of Gutman and Gutman (1940) when they were repeated the following day, although subsequently this was shown to be due to the poor precision of the method and its susceptibility to contamination by trace materials adsorbed on glassware. The present work shows (Fig. 2) that, provided the serum is promptly refrigerated, no other treatment is necessary; failing this, quite rapid inactivation occurs which is prevented by acidification but not by detergent. Once acidified, the serum may be kept several days at room temperature—certainly long enough to permit mailing of the sample to a central laboratory. Our findings regarding the stability of untreated serum are in good agreement with those of an earlier study (King and Jegatheesan, 1959).

#### CLINICAL VALUE OF THE ASSAY

The significance of raised serum AcPase activity has been discussed by many authors. It has become established as a fact of laboratory medicine that when carcinoma of the prostate metastasizes to bone raised and indeed sometimes very high levels of AcPase are found in the serum of most but not by any means all patients (Gutman and Gutman, 1938; Woodard, 1959). These earlier workers on the whole agree that raised levels occur in about half the cases where the metastases are to soft tissues only, and they are uncommon when the tumour remains within the confines of the prostatic capsule. Most of the recent reports based on fairly large surveys support this consensus (Veterans Administration, Cooperative Urological Research Group, 1968; Murphy *et al*, 1969; Prout, 1969; Amador *et al*, 1969; Marshall and Amador, 1969). Indeed the point has been made

by Marshall and Amador (1969) that no single case in their series was diagnosed on the basis of AcPase activity; in all their cases with raised values the diagnosis had already been reached by the clinician before the result of the estimation was known. The traditional view regarding the value of AcPase determinations in following the progress of the disease, succinctly summarized by Woodard (1959), has also been thrown in doubt (Schwartz, Greenberg, and Bodansky, 1963; Prout and Brewer, 1967). It has, moreover, been shown by Mobley and Frank (1968) that anaplastic tumours neither produce nor secrete AcPase and, even in the presence of bone metastases, are usually associated with normal levels.

The method presented appears to be quite sensitive in detecting lesions before they reach the stage of bone metastases. Against this must be set the higher incidence of elevations among patients with other prostatic abnormalities or even apparently normal prostates, although the influence of prostatic massage is known in some of these cases and suspected in others. It is also very difficult to exclude a small and clinically undetectable lesion in these patients who did not come to operation, except by careful follow up. At the present there is no method of choice for AcPase determination, but the evidence of this report suggests that the method described is worthy of serious consideration by those laboratories starting to develop the test, or unhappy with their current method.

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