A METHOD FOR ESTIMATING SERUM ACID PHOSPHATASE OF PROSTATIC ORIGIN*

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In 1949, Abdul-Fadl and King (1) reported an extensive study on the properties of acid phosphatases of erythrocytes, liver, spleen, and prostate of normal human subjects. It was established that cupric ions and formaldehyde inhibited erythrocyte but not prostatic acid phosphatase, whereas L-tartrate inhibited prostatic but not erythrocyte acid phosphatase.

In the present study, the ability has been tested of L-tartrate to inhibit the "prostatic"[1] component of the serum acid phosphatases.

Method

Principle—The hydrolysis of phenyl phosphate is measured in the absence and in the presence of L-tartrate. The difference found in the amount of phenol liberated is believed to represent the inhibition of acid phosphatase of prostatic origin. The analytical procedure is based on the modification by Benotti *et al.* (2) of the Gutman and Gutman (3) version of the King-Armstrong method (4).

Reagents—

Citrate buffer, pH 4.9. Dissolve 18.9 gm. of citric acid (Merck, c.p.) in 500 cc. of H_2O and add 180 cc. of 1 N NaOH and 100 cc. of 0.1 N HCl. Make up to 1 liter with distilled H_2O. Check the pH of the buffer and adjust to pH 4.9 with 1 N NaOH or 0.1 N HCl, as needed. Store in the refrigerator in a glass-stoppered bottle.

Substrate, disodium phenyl phosphate (Paul-Lewis Laboratories, Madison, Wisconsin). A 1 per cent aqueous solution of substrate is prepared

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[1] "Prostatic" is intended to indicate that portion of the serum acid phosphatase which is inhibited by L-tartrate under the experimental conditions defined in this paper.

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fresh every 2 weeks in routine work or when the blank contains more than 0.015 mg. of phenol.

Dilute phenol reagent, Folin and Ciocalteu (prepared solution, Mahady and Company, Boston). Dilute 1 volume with 2 volumes of water. Store in the refrigerator in a brown glass bottle.

Sodium carbonate (anhydrous, Merck reagent). A 20 per cent aqueous solution is prepared. Filter daily before using.

Standard aqueous phenol solution containing 0.01 mg. per cc. Prepare a solution of phenol in 0.1 N HCl which contains approximately 1 mg. per

TABLE I

"Prostatic" Phosphatase in Blood Serum of Normal Males

The results are expressed in King-Armstrong units per 100 cc.

Subject	Age	Series 1		Series 2*	
		Total phosphatase	"Prostatic" phosphatase	Total phosphatase	"Prostatic" phosphatase
	yrs.				
D	35	1.2	0.3	1.0	0.4
B	28	1.0	0.2		
DO	37	1.1	0.2	0.6	0.1
H	36	1.0	0.1		
S	40	1.0	0.1	1.0	0.1
K	45	1.3	0.2	0.4	0.3
R	40	1.6	0.1		
J	30	1.5	0.1	0.9	0.2
ST	55	1.7	0.3		
C	21	1.1	0.2		
Q	29	1.1	0.1	0.7	0.2
G	22	0.7	0.1	0.8	0.1
HE	29	0.7	0.2		

* The data under Series 2 were collected at an interval of 1 month or so after the data of Series 1.

cc. of "crystallized phenol" (Mallinckrodt analytical reagent). Transfer 25 cc. of this solution to a 250 cc. Erlenmeyer flask, add 50 cc. of 0.1 N sodium hydroxide, and heat to 65° on a hot-plate. To the hot solution, add 25 cc. of 0.1 N iodine solution; stopper the flask and let stand at room temperature for 30 to 40 minutes. Add 5 cc. of concentrated HCl and titrate the excess iodine with 0.1 N thiosulfate solution. Each cc. of 0.1 N iodine (cc. of iodine added minus cc. of thiosulfate used in titration) corresponds to 1.567 mg. of phenol. On the basis of the titration data dilute the phenol solution with 0.1 N HCl so that 1 cc. contains 0.01 mg. of phenol.

0.2 M L-tartrate. 3.002 gm. of tartaric acid (c.p., Baker's "analyzed" or

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Merck's "reagent"; as supplied both are L(+)-tartaric acid) are dissolved in 50 cc. of H_2O. Add approximately 35 cc. of 1 N NaOH. Check and adjust pH to 4.9 and make up to 100 cc. Store in the refrigerator in a glass-stoppered bottle.

Apparatus—Coleman junior spectrophotometer.

TABLE II

Distribution of "Prostatic" and Total Acid Phosphatase Values

	No. of male patients					
	Units of "prostatic" phosphatase per 100 cc. serum					
	0	0.1	0.2	0.3	0.4	0.5
Series 1, No. of patients	3	26	38	11	6	2
" 2, " " "	3	30	19	8	4	1
	Units of total acid phosphatase per 100 cc. serum					
	0–0.5	0.6–1.0	1.1–1.5	1.6–2.0	2.1–3.0	
Series 1, No. of patients	11	26	34	6	2	
" 2, " " "	8	36	16	3	2	
	No. of female patients					
	Units of "prostatic" phosphatase per 100 cc. serum					
	0	0.1	0.2	0.3	0.4	0.5
Series 1, No. of patients	17	25	14	4		2
" 2–4,* No. of patients	7	18	9	4	2	1
	Units of total acid phosphatase per 100 cc. serum					
	0–0.5	0.6–1.0	1.1–1.5	1.6–2.0	2.1–3.0	
Series 1, No. of patients	2	21	24	5	4	
" 2–4,* No. of patients	4	7	10	7	4	

* A number of patients were studied on two, three, and sometimes four different occasions. Since these results were in the same range of activity, they have been grouped together.

Procedure—Four digests are prepared in Pyrex test-tubes as follows: Digest A (serum blank), 10 cc. of H_2O and 0.5 cc. of serum; Digest B (substrate blank), 9 cc. of buffer, 0.5 cc. of H_2O, and 1.0 cc. of substrate; Digest C (total), 0.5 cc. of serum, 9 cc. of buffer, 1 cc. of substrate; Digest D (tartrate), 0.5 cc. of serum, 8 cc. of buffer, 1 cc. of 0.2 *M* L-tartrate, and 1 cc. of substrate. In all of these, substrate was the last addition.

These are well mixed, stoppered, and Digests B, C, and D are first

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brought to 38° in a beaker of water and are then placed in an incubator at 38° and the time recorded. Digest A is treated with 4.5 cc. of phenol reagent and filtered after 15 minutes. The length of incubation time is variable: 0.5 hour for values expected to exceed 20 King-Armstrong units, 1.0 hour for 6 to 19 units, and 3 hours for values expected to be lower than 6 units. The rate of hydrolysis is linear for periods of at least 3 hours. At the end of the incubation period, 4.5 cc. of phenol reagent are added separately to Digests B, C, and D. These are filtered 15 minutes after mixing.

TABLE III

"Prostatic" and Total Acid Phosphatase in Serum of Patients with Metastatic Prostatic Cancer

The results are expressed in units per 100 cc.

Patient	Total serum acid phosphatase	"Prostatic" serum acid phosphatase
A. W.	11.6	10.2
G. H.	20.6	18.2
A. C.	6.8	4.0
C. E.	5.1	2.5
J. H.	32.2	27.9
C. F.	46.1	42.2
D. P.	8.8	7.1
C.,* Jan. 4	16.8	15.0
" Feb. 5	4.4	3.5
" " 25	1.8	1.3
" Apr. 1	1.0	0.6

* Patient C. was placed and maintained on stilbestrol therapy shortly after January 4, 1952.

Color development results after adding 2.5 cc. of 20 per cent Na_2CO_3 to 10 cc. of the filtrates of these four digests and incubating the mixtures at 38° for 5 minutes. With each day's determination a standard phenol digest is prepared (2.0 cc. of standard phenol (0.01 mg. per cc.), 5 cc. of H_2O, 3 cc. of phenol reagent) and a reagent blank (7 cc. of H_2O, 3 cc. of phenol reagent), and their colors are developed with 2.5 cc. of 20 per cent Na_2CO_3 so as to be read along with Digests A, B, C, and D.

Immediately after the 5 minute interval, the optical density of each of these solutions is measured in a Coleman junior spectrophotometer at 660 mμ after adjusting the machine to zero optical density with the reagent blank.

Calibration Curve—Standard solutions are prepared with increasing amounts of the phenol standard (0.01 mg. per cc.). Each tube, in addition to the standard, contains 3.0 cc. of phenol reagent and water to

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adjust the volume to 10 cc. To each tube are added 2.5 cc. of 20 per cent Na_2CO_3 solution. After 5 minutes incubation at 38°, the optical density is measured, as described above, including the incubated reagent

TABLE IV

Effect of L-*Tartrate on Serum Acid Phosphatase with and without Added Prostatic Acid Phosphatase*

Patient	Serum acid phosphatase, units per 100 cc.						
	Total	Plus L-tartrate	"Prostatic"	Serum + prostatic acid phosphatase*	Mixture + L-tartrate	"Prostatic" found	"Prostatic" calculated†
Female patients							
S.	1.5	1.4	0.1	27.0	1.7	25.3	25.6
M.	4.6	4.2	0.4	30.6	3.9	26.7	26.2
F.	1.5	1.4	0.1	12.2	1.6	10.6	10.8
C.	2.4	2.1	0.3	12.6	2.2	10.4	10.5
Cu.	1.0	0.8	0.2	11.1	0.7	10.4	10.3
K.‡	7.5	7.4	0.1	17.1	7.4	9.7	9.7
Co.	1.4	1.3	0.1	9.0	1.3	7.7	7.7
T.	2.1	1.8	0.3	10.7	1.4	9.3	8.9
F.	1.0	1.0	0.0	9.6	1.0	8.6	8.6
B.	1.1	1.0	0.1	12.3	1.0	11.3	11.3
Mc.	1.0	0.9	0.1	10.8	1.0	9.8	9.9
Male patients without cancer of prostate							
T.	1.4	1.2	0.2	13.5	1.3	12.2	12.3
R.	1.1	0.9	0.2	11.4	1.1	10.3	10.5
F.	1.1	0.9	0.2	12.8	1.1	11.7	11.9
N.	1.1	0.9	0.2	9.0	1.5	7.5	8.1
K.	0.5	0.4	0.1	8.4	0.5	7.9	8.0
G.	0.7	0.6	0.1	9.9	0.6	9.3	9.3
W.	1.4	1.1	0.3	12.6	1.2	11.4	11.5
O.	0.9	0.5	0.4	11.1	0.6	10.5	10.6
Wa.	1.0	0.8	0.2	22.2	0.7	21.5	21.4
Wh.	1.1	0.9	0.2	21.6	1.1	20.5	20.7
O'H.	0.7	0.6	0.1	25.5	0.7	24.8	24.9
C.	0.7	0.5	0.2	28.2	0.7	27.5	27.7
V.	0.7	0.6	0.1	12.6	0.6	12.0	12.0
P.	0.5	0.3	0.2	10.8	0.2	10.6	10.5
Y.	1.5	1.0	0.5	8.7	0.6	8.1	7.7
M.	0.2	0.1	0.1	9.6	0.1	9.5	9.5
Ca.	1.0	1.1	0.0	10.2	1.0	9.2	9.1
J.	1.6	1.3	0.3	10.2	1.3	8.9	8.9
B.	2.1	1.7	0.4	10.2	1.7	8.5	8.5
Q.	1.1	1.0	0.1	14.7	1.0	13.7	13.7

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TABLE IV—*Concluded*

Patient	Serum acid phosphatase, units per 100 cc.						
	Total	Plus L-tartrate	"Prostatic"	Serum + prostatic acid phosphatase*	Mixture + L-tartrate	"Prostatic" found	"Prostatic" calculated†
Patients with cancer of prostate							
C.,§ Jan. 21	28.2	1.8	26.4	37.8	2.0	35.8	36.0
" Feb. 25	1.8	0.5	1.3	11.7	0.6	11.1	11.2
" July 3	0.9	0.8	0.1	10.8	1.2	9.6	10.0
" " 7	0.7	0.4	0.3	8.7	0.4	8.3	8.3
H., July 3	38.4	3.2	35.2	41.4	2.8	38.6	38.2
" " 8	37.2	2.2	35.0	42.0	3.4	38.6	39.8
D., " 2	37.8	3.0	34.8	49.2	2.7	46.5	46.2
" " 7	33.6	1.6	32.0	37.8	1.9	35.9	36.2

* Highly purified human prostatic acid phosphatase (10,000 units per 100 cc.) was kindly supplied by Dr. Gerhard Schmidt. 0.2 cc. of a 1:200 dilution of acid phosphatase was mixed with 1.8 cc. of serum. 0.5 cc. aliquots were assayed as described.

† These figures were obtained by subtracting from the value in the fifth column (serum + prostatic acid phosphatase) the value found for the same patient under the third column (+ L-tartrate).

‡ Cancer of the breast with bone metastases.

§ See foot-note to Table III.

blank. The concentration of phenol is plotted against optical density to yield a straight line. This curve is readily reproducible.

Calculation—The optical density obtained with Digests A, B, C, and D is substituted in the calibration curve to yield the amount of phenol in mg. present in each digest.[2]

$$(1)\qquad \frac{\text{C mg.} - (\text{A mg.} + \text{B mg.})}{\text{Hrs. of incubation}} \times \frac{15}{10} \times \frac{100}{0.5} = T \text{ units per 100 cc. serum}$$

$$(2)\qquad \frac{\text{D mg.} - (\text{A mg.} + \text{B mg.})}{\text{Hrs. of incubation}} \times \frac{15}{10} \times \frac{100}{0.5} = U \text{ units per 100 cc. serum}$$

(3) T units $-$ U units = units of "prostatic" acid phosphatase per 100 cc. serum

The units defined here are equivalent to the number of mg. of phenol (King-Armstrong units) (4). Whenever possible, Digests B, C, and D have been analyzed in duplicate.

EXPERIMENTAL

Normal Male Subjects—Venous blood specimens were collected from volunteers between the ages of 21 and 55 years, and both total and "pros-

[2] T represents total; U represents uninhibited serum acid phosphatase.

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tatic" serum acid phosphatases were determined in duplicate. Some months later, whenever possible, these determinations were repeated, yielding two series of analyses. The results are listed in Table I.

Patients without Prostatic Cancer—These patients, male and female, hospitalized for chronic disease, including cancer, cardiovascular disorders, arthritis, diabetes, etc., were studied in the same manner as the normal male subjects (Table II).

TABLE V

Comparison of Effect of Cu^{++} and L-Tartrate Added to Serum on Acid Phosphatase Activity

Material	Acid phosphatase, units per 100 cc. serum			
	Experiment No.	Total	L-Tartrate "prostatic"	Cu^{++}-resistant*
Purified prostatic acid phosphatase†	1	20.1	19.7	20.1
	2	13.8	13.5	13.8
Serum + prostatic acid phosphatase	1	30.6	26.7	29.7
	2	11.1	10.4	10.8
	3	11.1	10.5	10.5
Serum	1	26.1	23.0	23.9
	2	1.1	0.1	0.9
	3	0.8	0.2	0.7
	4	1.3	0.2	1.1
	5	1.6	0.3	1.2
	6	1.2	0.1	1.2
	7	1.5	0.1	1.5
	8	1.6	0.0	1.3
	9	0.8	0.0	0.8
	10	1.8	0.8	1.7
	11	1.2	0.0	1.2
	12	0.7	0.1	0.7

* Total acid phosphatase determination in the presence of 0.0002 M $CuSO_4$ final concentration.

† See foot-note to Table IV.

Patients with Prostatic Cancer—Values obtained on patients for total and "prostatic" acid phosphatases are given in Table III.

Effect of L-Tartrate on Serum Enriched with Prostatic Acid Phosphatase—These results appear in Table IV.

Comparison of Effects of Cu^{++} and L-Tartrate on Serum Acid Phosphatase—See Table V.

DISCUSSION

The need exists for a method of determining selectively that portion of the serum acid phosphatase which is of prostatic origin. This is par-

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ticularly important in the differential diagnosis of cancer of the prostate in patients exhibiting normal serum acid phosphatase values.

Two factors mainly have been responsible for the existence of a category of serum acid phosphatase levels intermediate between those characteristic of healthy men and of patients with prostatic cancer. One is the phenomenon of hemolysis, which, however slight it may be, enriches the serum with erythrocyte acid phosphatase. The other is the failure to correct for a certain degree of spontaneous hydrolysis, which the substrate, phenyl phosphate, depending on its purity, may undergo during the incubation of the serum digest. In the past, these difficulties have been overcome separately by the formaldehyde inhibition of erythrocyte acid phosphatase (5, 6) and by the use of suitable substrate controls in the assay procedure (2).

The method described in this paper is expected to contract markedly the range of clinically equivocal values for serum acid phosphatase and, in addition, to permit a reasonably reliable estimation of concentration of serum acid phosphatase of prostatic origin. Thus, L-tartrate has been found (1) to inhibit prostatic and not erythrocyte acid phosphatase, and hence the factor of hemolysis becomes unimportant. The extent of spontaneous hydrolysis of the substrate is measured and suitable corrections are made, as described by Benotti *et al.* (2). Both of these advantages have been incorporated in the present method.

The view that, in males, the serum acid phosphatase inhibited by L-tartrate is most probably of prostatic origin is based on the following observations. (1) Solutions of purified human prostatic acid phosphatase were almost completely inhibited by 0.02 M L-tartrate (1) (see also Table V, this paper). (2) All patients with active cancer of the prostate showed a high proportion of "prostatic" phosphatase in their sera. (3) L-Tartrate inhibited invariably "prostatic" phosphatase of the patients' sera, as well as added purified prostatic acid phosphatase (Tables III and IV). This was true in all thirty-nine patients studied with or without cancer of the prostate. (4) In patients with cancer of the prostate in which the disease had become activated,[3] the "prostatic" acid phosphatase component of the serum increased progressively to high levels, forming an increasingly greater proportion of the serum acid phosphatases.

In chronically ill subjects without evidence of cancer of the prostate, as well as in healthy men, uniformly low serum "prostatic" phosphatase values were found. It would appear that the majority of these subjects show 0.1 to 0.3 units per 100 cc. of serum "prostatic" enzyme, 0.5 unit per 100 cc. representing the upper limit of normal in this series.

It is not surprising to find that small amounts of "prostatic" phospha-

[3] Fishman, W. H., and Homburger, F., unpublished data.

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tase are present in the sera of women, since acid phosphatase arising from tissues other than prostate (1) is inhibited by tartrate. It is clear that a much larger percentage of women than men show no trace of "prostatic" enzyme. Moreover, the large number of heterogeneous patients studied in this paper has failed to reveal a single disease entity, other than prostatic cancer, which is associated with an elevated "prostatic" phosphatase in serum. It would appear reasonable to assume, therefore, on the basis of the present evidence, that an elevated serum "prostatic" phosphatase in male subjects is indeed of prostatic origin.

Although the results obtained in the presence of Cu^{++} (Table V) agreed well with the "prostatic" phosphatase figures at high serum acid phosphatase values, poor agreement was seen in low phosphatase sera. Accordingly, attempts to utilize the action of Cu^{++} for selectively estimating serum acid phosphatase of prostatic origin were abandoned.

SUMMARY

A method is described which measures the extent of inhibition of serum acid phosphatase by L-tartrate. Experimental evidence is presented which supports the view that, in male subjects, the greatest part of this inhibited fraction of serum acid phosphatase is most likely of prostatic origin.

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