Properties of the Acid Phosphatases of Erythrocytes and of the Human Prostate Gland

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There is much evidence indicating the non-identity of acid phosphatases of different origin. Davies (1934) has shown that the acid phosphatase of the red cell differs from that of spleen in that the former hydrolyses α -glycerophosphate much more readily than the β -compound, while with the spleen enzyme β -glycerophosphate is hydrolysed more quickly. Kutscher & Worner (1936) described the irreversible inactivation of the prostatic acid phosphatase by certain narcotics, including alcohols. This was used by Herbert (1944, 1945, 1946) for the identification and determination of the prostatic enzyme in blood serum, whose normal acid phosphatase is hardly affected by ethanol treatment. King, Wood & Delory (1945), on the other hand, found the acid phosphatases of prostate and red cells to be similar in many respects, including easy destruction by ethanol, but formaldehyde and L-tartrate (Abul-Fadl & King, 1947, 1948*a, b*) sharply distinguish the two enzymes by complete inactivation of the one or the other. In the present investigation the question of the identity of these two acid phosphatases is further dealt with. For this purpose, the rates of hydrolysis of phenyl phosphate by each enzyme at different pH values were determined, together with the relative rates of hydrolysis of α - and β -glycerophosphates. This was followed by studies of the effects of organic and inorganic substances belonging to different groups of a possible activating or inhibiting nature. The nature of the enzymes is discussed in the light of these experiments.

EXPERIMENTAL

Enzyme solutions

Normal fresh human prostate glands were decapsulated, cut into fine pieces and ground with sand in a mortar with 5 times their weight of 0.9% NaCl solution. A few drops of toluene were added and the mixture was left at room temperature to autolyse for 2–3 days. The mixture was then filtered and highly diluted (e.g. 1000–5000 times) before determining its activity.

Freshly drawn samples of blood from man, ox, sheep, pig, rabbit, rat and guinea pig were centrifuged, the plasma with the top layer of white cells separated, and the red cells twice washed with 0.9% NaCl by spinning and decantation. Washed red cells (1 ml.) were lysed in 9 ml. water and the solution centrifuged. The clear supernatant was used immediately for determining the enzyme activity.

Determination of pH-hydrolysis curves

Acetate buffer solutions were prepared to cover the range of pH 3.6–6.0. The pH of each solution was determined by the indicator method and by the glass electrode (Muirhead) after mixing with an equal volume of the 0.02 M-disodium phenyl phosphate substrate solution and warming the mixture to 37° in a water bath. The hydrolysis period was exactly 30 min. at 37°, when the liberated phosphorus or phenol was determined.

Activating and inhibiting substances

Metallic ions, acid radicals and amino-acids. The effects of the following substances on the rates of enzymic hydrolysis of phenyl phosphate by the prostatic and the red-cell acid phosphatases have been investigated: Ca^{++} , Co^{++} , Cr^{+++} , Cu^{++} , Fe^{+++} , Mn^{++} and Zn^{++} ; arsenate, citrate, cyanide, formate, fluoride, oxalate, salicylate, tartrate and tauroglycocholate; glycine, alanine, cysteine and glutathione. Solutions of the pure substances (A.R. whenever possible) were prepared so that 1 ml. of each solution, when mixed with buffer, substrate and enzyme solutions, would furnish the appropriate concentration of the substance in the final mixture. Acetate or citrate buffers of pH 5.0 were used. The liberated phenol was determined as an index of activity, except when there was a direct interference by certain metals or other substances with the method of phenol estimation. In such cases the liberated phosphate was determined either by the King (1932) method or by the Allen (1940) amidol method, and acetate was used instead of citrate buffer which interferes with the determination of free phosphate.

Iodoacetic acid. Neutral solutions containing 5–10 mg. of iodoacetate were mixed with the enzyme solutions and the activities were determined with and without previous incubation.

Stilboestrol (4:4'-dihydroxy- α - β -diethylstilbene). Tablets containing 5 mg. stilboestrol were ground in a mortar and thoroughly extracted with dry chloroform. The clear filtered chloroform extract was adjusted to a concentration of 1 mg. stilboestrol/ml.

The effect of this substance on prostatic and red-cell phosphatases was studied in two ways: (a) by incubating the enzyme solution with stilboestrol for 1 hr. at room temperature, and comparing its activity with that of untreated solution, which was similarly incubated. For this purpose 5 ml. stilboestrol solution in chloroform (1 mg./ml.) were pipetted into a 100 ml. measuring flask and evaporated by gentle warming in a water bath. The flask was then filled with the enzyme solution, and kept at room temperature for 1 hr. with gentle rotation to assure saturation and equal distribution of the stilboestrol. (b) By including 0.5 mg. stilboestrol in the buffer-substrate-enzyme mixture.

In every case, chloroform was completely eliminated before mixing with the enzyme. This is especially important in the case of the prostatic phosphatase which is sensitive to this substance. Vigorous shaking was avoided, since the prostatic enzyme is rapidly inactivated, especially in dilute solutions.

RESULTS

The pH-hydrolysis curve indicates the presence of two acid phosphatases in the red cells of man and other animals; a 'first acid enzyme' with optimum

temperature for a few hours. In some species the two optima lie well apart from each other and are fairly sharp (e.g. human, rabbit, ox and sheep, Fig. 1). In other species, however, the two optima are so close to each other, and that of the second enzyme (of higher pH optimum) is so broad that they are difficult to determine (e.g. pig and rat, Fig. 2).

Fig. 1. pH-Hydrolysis curves for the acid phosphatases of the red cells. Solid line without added Mg^{++} ; broken line, with added 0.01 M- Mg^{++} .

pH of 4.3–4.8 and a 'second acid enzyme' optimally active at pH 5.0–5.7. The optimum for each enzyme was found to vary a little, not only from one species to another, but even in different individuals of the same animal species. The first acid enzyme is very labile and can no longer be detected in enzyme solutions which have been left to stand at room

Fig. 2. pH-Hydrolysis curves of 0.01 M-phenyl phosphate by red-cell acid phosphatases. Solid line, without added Mg^{++} ; broken line, with added 0.01 M- Mg^{++} .

Fig. 3. pH-Hydrolysis curve of 0.01 M-phenyl phosphate by prostatic acid phosphatase. Solid line, without added Mg^{++} ; broken line, with added 0.01 M- Mg^{++} .

Magnesium inhibits the phosphatases of the red cells especially in the more acid range. In some cases, where the enzyme activity was still marked at pH 6.0, magnesium was either without effect or showed slight activation; but in every case there was pronounced inhibition at the optimal pH.

Fig. 3 shows the shape of the pH-hydrolysis curve of phenyl phosphate by prostatic acid phosphatase. There is only one clearly defined optimum, at pH 5.0–5.2. Magnesium, at different pH values, was

either without effect or produced inhibition only near the optimum. The absence of marked inhibition at the more acid pH, which was very pronounced in the case of the phosphatases of the red cells, confirms the homogeneity of the prostatic enzyme.

Table 1 shows the relative rates of hydrolysis of α - and β -glycerophosphates and phenyl phosphate by the acid phosphatases of the prostate and red cells. β -Glycerophosphate is very slowly hydrolysed by the red-cell enzyme. The α -isomer is more quickly hydrolysed and phenyl phosphate much more rapidly. The very low activity of red-cell phosphatases against β -glycerophosphate has been repeatedly confirmed with red cells from different

β -glycerophosphates, but it seems to inhibit very slightly the hydrolysis of phenyl phosphate (though not as much as it does with the red-cell enzyme).

Table 2 shows the effect of certain metallic ions on the acid phosphatases of plasma, prostate and red cells. In acetate buffer calcium inhibits the red-cell enzyme slightly and the prostatic enzyme more strongly. (In citrate buffer, however, these inhibitory effects completely disappear owing to the formation of calcium complexes with the citrate.) Chromium, cobalt, manganese and zinc all have variable inhibitory effects on prostatic and red-cell phosphatases, and none was found to exhibit the slightest activating property.

Table 1. *Relative rates of hydrolysis of α - and β -glycerophosphates and phenyl phosphate by the acid phosphatases of the prostate and red cells*

(Acetate buffer pH 5; 30 min. at 37°.)

Enzyme solution	Hydrolysis (mg.P/30 min./100 ml. enzyme solution)								
	0.02M- β -Glycerophosphate			0.02M- α -Glycerophosphate			0.005M-Phenyl phosphate		
	Without Mg	0.003M-Mg	0.01M-Mg	Without Mg	0.003M-Mg	0.01M-Mg	Without Mg	0.003M-Mg	0.01M-Mg
Red cells (human)	0.2	0.25	0.3	11	11	11	55	—	48
Red cells (human)	0.2	0.2	0.25	9.2	9.2	—	43	37	—
Prostate in normal saline	29	29	30	28	28	—	53	—	50

Table 2. *Effect of metallic ions on the acid phosphatases of plasma, prostate and red cells*

(Sodium phenyl phosphate substrate; acetate buffer, pH 5; 30 min. at 37°. Activity expressed as 'units', i.e. mg. P/30 min./100 ml. enzyme solution.)

Enzyme solution	Control enzyme without inhibitor (units/100 ml.)	Percentage inhibition					
		Calcium (0.01M)	Chromium (0.01M)	Cobalt (0.01M)	Manganese (0.01M)	Nickel (0.01M)	Zinc (0.001M)
Red cells (human)	80	14	25	0	15	—	20
Red cells (human)	47	17	17	4	15	23	23
Red cells (rat)	88	10	20	—	17	—	—
Plasma (normal)	4.2	0	—	0	10	28	0
Prostate in normal saline	50	40	8	0	4	11	40
Seminal fluid	160	50	9	—	7	21	52
Prostate in normal plasma	10.0	16	—	0	0	4	12

sources and with different dilutions of enzyme, which were incubated with this substrate and acetate buffers at pH values from 3.6 to 6.6 for various lengths of time. (The glycerophosphate was a crystalline sample (Boots Pure Drug Co. Ltd.), and was rapidly hydrolysed by the prostatic enzyme, as well as by several alkaline phosphatases.) Magnesium at different concentrations activates very slightly the hydrolysis of β -glycerophosphate while practically not affecting that of the α -isomer. It invariably inhibits the splitting of the phenyl ester.

The prostatic enzyme, on the other hand, hydrolyses the β -glycerophosphate almost at the same rate as the α -ester, while the phenyl ester is hydrolysed about twice as fast. The effect of magnesium (0.003 and 0.01M) is almost negligible with the α - and

Table 3 shows the effect of copper and iron on the acid phosphatases of the prostate and red cells. That the nature of the buffer is of considerable importance in the study of the effect of metals is also clearly shown. Copper in concentrations as low as 0.0002M is a strong inhibitor of the red-cell phosphatases (about 90 % inhibition) in either acetate or citrate buffer media. With the prostatic enzyme copper in acetate causes slight inhibition (about 8 % with 0.0002M-CuSO₄); with citrate buffer, on the other hand, a marked activation is noticed with 0.001 and 0.0005M-copper sulphate.

In an acetate buffer the iron present is in the form of a colloidal basic iron acetate. This causes a serious inhibition of the prostatic enzyme (80–90 %) and a less marked inhibition of the red-cell phosphatases

Table 3. *Effect of copper and iron on the acid phosphatases of prostate and red cells in presence of acetate and citrate buffer solutions*

(Disodium phenyl phosphate substrate; 30 min. at 37°. Activity expressed as mg. P/30 min./100 ml. enzyme solution.)

Enzyme solution	Enzyme activity without inhibitor (units/100 ml.)	Percentage inhibition in presence of copper			Percentage inhibition in presence of iron	
		0.001 M	0.0005 M	0.0002 M	0.001 M	0.0005 M
Acetate buffer pH 5.0						
Red cells (human)	53	96	96	95	25	9
Red cells (human)	47	100	—	—	25	9
Red cells (human)	80	90	88	85	18	5
Prostate in normal saline	50	22	10	—	89	—
Prostate in plasma	60	42	18	8	78	78
Citrate buffer pH 5.0						
Red cells (human)	47	99	94	90	6	0
Prostate	145	(15 A.)*	(8 A.)*	0	0	0
* A. = Activation.						

* A. = Activation.

Table 4. *Effect of glycine, alanine and cysteine on the acid phosphatases of prostate and red cells*

(Acetate buffer pH 5; 0.005 M-phenyl phosphate substrate; 30 min. at 37°.)

Hydrolysis (mg. P/hr./100 ml. enzyme solution)

Enzyme solution	Control (nothing added)	Glycine (M)				Alanine (M)				Cysteine (M)			
		0.02	0.01	0.005	0.0001	0.02	0.01	0.005	0.0001	0.02	0.01	0.005	0.001
Red cells (human)	22	23	23	22	22	—	—	—	—	—	—	—	—
Red cells (rat)	40	—	—	—	—	—	—	—	—	36	40	42	—
Red cells (human)	28	28	29	29	29	28	28	28	30	27	30	30	30
Red cells (human)*	29	29	29	30	29	29	30	30	30	27	29	31	30
Prostate in normal saline	41	36	37	40	42	37	39	40	42	42	45	43	43
Prostate in normal saline*	40	36	37	39	39	—	—	—	—	37	43	44	42
Seminal fluid in normal saline	29	—	—	—	—	—	—	—	—	26	31	—	34

* After 3 days' dialysis in cellophan bag at 6° against running distilled water.

(10–25 %). In citrate buffer medium, however, the pronounced inhibitory action on the prostatic enzyme is completely annulled; while the inhibition of the red-cell enzyme is still marked with 0.001 M-concentration, but is abolished when the concentration is reduced to 0.0005 M.

Table 4 shows the effect of glycine, alanine, and cysteine on the phosphatases of prostate and red cells. The concentrations of glycine and alanine, which have been reported to activate the alkaline phosphatases, do not exhibit such an effect on the acid enzymes. Higher concentrations of these amino-acids have no effect on the red-cell enzymes, but slightly inhibit the prostatic phosphatase. Cysteine in 0.02 M-concentration produces a slight inhibition with red-cell and prostatic enzymes; 0.01 M-concentration is either without effect or slightly activates both enzymes. After prolonged dialysis of acid phosphatases, there was still no change in response towards amino-acids.

Table 5 shows the effect of cysteine and glutathione on the copper-inhibited phosphatases of the red cells. When cysteine or glutathione was added, together with copper, to the enzyme solution, the latter did not inhibit the enzyme markedly. But if copper was

Table 5. *Effect of cysteine and glutathione on the copper-inhibited phosphatases of the red cells*

(Acetate buffer, pH 5; 0.005 M-phenyl phosphate substrate; 1 hr. at 37°; two experiments, A and B.)

	Hydrolysis (mg. P./hr./100 ml. enzyme solution)	
	A	B
Red-cell solution alone	45	19
Red-cell solution with 0.01 M-cysteine	46	17
Red-cell solution with 0.01 M-glutathione	46	18
Red-cell solution with 0.0005 M-CuSO ₄	8	2
Red-cell solution with 0.02 M-cysteine + 0.0005 M-CuSO ₄ both added at the same time	36	15
Red-cell solution incubated with 0.0002 M-CuSO ₄ for 15 min; activity determined in presence of 0.02 M-cysteine	8	2
Same experiment repeated, substituting cysteine by 0.02 M-glutathione	8	2

allowed to react with the enzyme first, the enzyme was inhibited, and it did not seem possible to reverse this inhibition with excess of either cysteine or glutathione.

Table 6 shows the effect of certain acid radicals. Arsenate strongly inhibited (about 80 %) the red-cell phosphatases and also inhibited by about 70 %

acids (hydroxy, ketonic, dicarboxylic and ethylenic) was tested. None was even nearly as effective as tartaric acid.

On trying the effect of the different stereoisomers of tartaric acid, however, the inhibitory action was found to be exhibited only by the L*- and the DL-racemic forms, not by the D- or the meso form.

Table 6. *Effect of acid radicals on the acid phosphatases of plasma, prostate and red cells*

(Acetate buffer, pH 5; phenyl phosphate substrate; liberated phosphate determined after 30 min. at 37°. The figures represent the average percentage of activation (+) or inhibition (-) effected in several experiments.)

Acid radical (0.01 M)	Activation or inhibition				
	Normal plasma (%)	Human red cells in water (%)	Prostate in normal saline (%)	Prostate in normal plasma (%)	Seminal fluid in normal saline (%)
Arsenate	-72	-80	-66	-70	-66
Citrate	0	+5	+8	+5	+6
Cyanide	0	+8	+12	+10	+15
Fluoride	-30	-8	-96	-96	-97
Formate	0	0	0	0	0
Oxalate	-30	-27	-22	-25	-25
Salicylate	0	0	0	0	0
Tartrate (L-)	0	0	-94	-95	-93
Tauroglycocholate	0	-77	-76	-85	-80

the enzymes of plasma and prostate. There is a slight activation of the red-cell and prostatic enzymes by the citrate radical, while the formate and the salicylate radicals are without any effect. Fluoride in 0.01 M-concentration has comparatively little effect (8-10 % inhibition) on the red-cell phosphatases, while exhibiting more inhibition (28 %) of plasma phosphatase, and very marked (more than 95 %) inhibition of the prostatic enzyme. Cyanide (0.01 M) was found to be either without effect or to have mild activating properties (up to 15 %) on both red-cell and prostatic acid phosphatases. Oxalate (0.01 M) inhibited the plasma, prostatic and red-cell phosphatases nearly to the same extent (about 25 %). Bile salts in the form of sodium tauroglycocholate (used in a final concentration of 0.24 % in buffer-substrate-enzyme mixture) strongly inhibited both prostatic and red-cell enzymes (70-90 %). The normal plasma phosphatase, on the other hand, did not seem to be affected. Samples of pathological plasma were also examined. Some of them showed slightly raised acid-phosphatase values in cases of liver disease, and all were found not to be affected by this bile salt.

Tartrate, while having no effect whatever on the acid phosphatase of either red cells or normal plasma, was found to be a very strong inhibitor of the prostatic enzyme (more than 90 % inhibition in 0.01 M-concentration).

Table 7 shows the effect of certain organic acid radicals, which are structurally related to tartaric acid, on the phosphatases of plasma, prostate and red cells. A series of aliphatic and aromatic organic

Dextrorotatory substances like dextrose have been tried also, as well as glycerol, pinacol, sorbitol,

Table 7. *Effect of organic acid radicals related to tartaric on the acid phosphatases of plasma, prostate and red cells*

(Citrate buffer, pH 5; 30 min. at 37°; liberated phenol was determined, except in the case of certain acids marked †, where the free phosphate was determined instead, and acetate buffer pH 5 was used in place of citrate. The acid solutions were adjusted to pH 5 before mixing with the buffer-substrate mixture. The figures represent the average percentage activation (+), or inhibition (-) effected.)

Acid (0.01 M unless otherwise stated)	Plasma	Red cells	Prostate
Lactic	0	0	0
Pyruvic†	+20	0	0
Malic	0	+8	+12
Maleic†	0	0	0
Malonic	0	0	0
Mucic	0	0	0
Fumaric	0	0	0
Succinic	0	0	0
Tartronic	0	0	0
D-Tartaric	0	0	0
DL-Tartaric	0	0	-92
meso-Tartaric	0	0	0
L*-Tartaric	0	0	-94
L-Tartaric (0.02 M)	0	0	-95
L-Tartaric (0.005 M)	0	0	-90
L-Tartaric (0.002 M)	0	0	-75
L-Tartaric (0.001 M)	0	0	-70

xylose and laevulose, but none produced an effect similar to L-tartrate.

* The L-form is the common dextrorotatory acid.

Table 8 shows that a quantitative determination of red-cell acid phosphatase, when present in admixture with the prostatic enzyme in serum or plasma, is a practical procedure, and the figures found are reasonably close to the calculated values. It is also possible by this means to demonstrate the presence of a tissue phosphatase, as distinct from the plasma and red-cell enzymes, in blood or in mixtures containing blood (Tables 8 and 11).

Table 8. Differential determination of red-cell and prostatic phosphatases in serum by means of L-tartrate

	Phosphatase (units/100 ml. solution)			
	Found		Calculated	
	Without tartrate	With 0.01M- L-tartrate	Red cell	Total
Normal serum	3.0	3.0	—	—
A, Prostate extract in normal serum	108	3.6	—	—
B, Red-cell solution in normal serum	66	66	—	—
A and B mixed in the following proportions:				
1 : 1	87	34	33	87
1 : 2	79	45	44	80
2 : 1	97	25	22	94

Table 9 shows that the inhibition of the prostatic enzyme by 0.005M-L-tartrate is reversible, and that the enzyme recovers its activity on dialysis. A control consisting of the enzyme without tartrate was set up, and similarly dialysed to safeguard against any extraneous change in the enzyme, either through dialysis or through incubation. Recovery was more rapid at room temperature than at 0°, but some inactivation results from incubation at room temperature.

Table 9. Reversal of L-tartrate inhibition of prostatic phosphatase by dialysis

	Activity (units/100 ml. enzyme solution)		
	Control (without tartrate)	Test (0.005M- tartrate)	Inhibition (%)
Start	120	15	88
After 24 hr. dialysis in cellophan at 5°	120	43	64
After further 24 hr. dia- lysis at room temperature	75	64	15

Fig. 4 shows the effect of L-tartrate on the acid phosphatases of red cells, prostate, liver and spleen at different pH values. Strong inhibition occurs with the last three enzyme preparations. With the

red cells inhibition is only detectable in the more acid part of the curve, while at the second optimum pH there is either no effect or slight activation. The alkaline phosphatases are slightly activated by L-tartrate.

Fig. 4. Effect of L-tartrate on the hydrolysis of phenyl phosphate by acid phosphatases. Solid line, without added L-tartrate; broken line, with added 0.01 M-L-tartrate.

Table 10 shows that the inhibitory effect of iodoacetic acid on the prostatic and the red-cell enzymes is quite evident even without previous incubation of this substance with the enzyme. Both enzymes are inhibited nearly to the same extent.

Table 11 shows the effect of certain other organic substances on acid phosphatases. Incubation with different alcohols, especially the lower homologues,

Table 10. *Effect of iodoacetic acid on the acid phosphatases of prostate and red cells*

(Acetate buffer, pH 5; 0.005 M-phenyl phosphate; 30 min. at 37°. Iodoacetic acid solution neutralized before mixing with the enzyme. Activity: mg. P/30 min./100 ml. enzyme solution.

Enzyme	Control without inhibitor (mg. P)	Iodoacetic acid		
		Amount added (mg.)	Activity (mg. P)	Inhibition (%)
Red cell (human)	39	5	16	59
Red-cell solution incubated with inhibitor for 30 min. at 6° at pH 7	39	5	9	77
	39	10	4	90
Prostate extract in normal saline	150	5	62	59
	150	10	40	73
Prostate extract incubated with inhibitor for 30 min. at 6° at pH 7	150	5	26	83

inhibits the acid phosphatases of bile, prostate, red cells and kidney, and slightly affects that of normal plasma. Acetone produces a similar effect. Formaldehyde, in 0.5 % concentration in the buffer substrate mixture, completely inhibits the red-cell phosphatases and partially destroys the plasma, liver,

Table 12. *Summary of the effect of different treatments on the acid phosphatases of prostate and red cells*

Treatment	Prostatic acid phosphatase	Red-cell acid phosphatase
Alcohol incubation	Inhibition 90–100%	Inhibition 70–80%
Copper (0.0002 M):		
Acetate buffer	Inhibition 8%	Inhibition 96%
Citrate buffer	No effect	Inhibition 90%
Cyanide	Slight activation	Slight activation
Cysteine	Slight activation	Slight activation
Formaldehyde (0.5%)	No effect	Inhibition 100%
Fluoride (0.01 M)	Inhibition 97%	Inhibition 10%
Hydrolysis of α - and β -glycerophosphoric esters	β slightly $> \alpha$	$\alpha > \beta$
Iron (0.0005 M):		
Acetate buffer	Inhibition 80%	Inhibition 9%
Citrate buffer	No effect (or slight activation)	No effect
Magnesium (0.01 M)	No effect (or slight inhibition)	Inhibition
Manganese	Slight inhibition	Slight inhibition

DISCUSSION

Roche, Thoai & Baudoin (1942) reported the presence of two phosphomonoesterases in the red cells of the ox and rat, optimally active at pH 4.6–4.8 and at pH 5–5.5 respectively. They added that the first enzyme is very unstable and is strongly inhibited by

Table 11. *Effect of certain organic substances on acid phosphatases*

(Citrate buffer, pH 5; phenyl phosphate. In the case of alcohols and acetone the enzyme was previously incubated with two-fifths its volume of the corresponding alcohol or acetone before determining its activity. The other substances were included in the buffer-substrate mixture during hydrolysis. The figures represent the average percentage inhibition.)

Enzyme	Inhibition (%)						
	Alcohol			Acetone	Formaldehyde (0.5%)	L-Tartrate (0.01 M)	Stilboestrol
	Methanol	Ethanol	Butanol				
Red cells	60	75	80	70	100	0	0
Prostate in normal saline	65	80	90	100	0	95	0
Prostate in normal plasma	100	100	100	100	0	95	0
Normal plasma	10	10	15	30	40	0	0
Liver:							
Human (adult)	—	0	—	0	35	70	0
Human (new born)	—	0	—	—	20	70	0
Bile	—	80	—	—	35	0	—
Kidney:							
Human (adult)	—	40	—	—	40	80	0
Human (new born)	—	50	—	—	20	85	0
Spleen:							
Human (adult)	—	0	—	—	40	70	0

kidney and spleen enzymes (20–60 %). The prostatic enzyme, on the other hand, is not affected to any extent under these conditions of treatment. Stilboestrol is shown to have no detectable effect on any of these tissue acid phosphatases.

magnesium and manganese, while the second acid enzyme is relatively stable and is activated by these two metals. The present work has confirmed these authors' claims as regards the presence of two acid phosphatases in the red cells of humans as well as other animals. The first enzyme has been found to be

Table 12 summarizes some of the above results.

very unstable and unless the blood is very fresh it may escape detection. It is much more strongly inhibited by magnesium than the second acid enzyme, and is sensitive to L-tartrate which does not affect the latter.

On the other hand, the presence of only one optimum in the pH-hydrolysis curve of phenyl phosphate by the prostatic enzyme has been proved.

The acid phosphatase of mammalian erythrocytes has been reported to be activated by magnesium (Jenner & Kay, 1931; Davies, 1934; Roche, 1931; Roche *et al.* 1942). Behrendt (1943), on the other hand, reported magnesium to be without effect on the phosphatases of human erythrocytes. Kutscher & Worner (1936) also found that magnesium has no effect on the prostatic enzyme. In the present investigation, magnesium has been found to activate slightly the hydrolysis of β -glycerophosphate by the red-cell enzyme but to exert no effect on the hydrolysis of the α -ester. On the other hand, it definitely inhibits the splitting of the phenyl phosphate. The prostatic enzyme, however, is either not affected by magnesium or only slightly inhibited. The fact that no conclusive evidence has been obtained concerning the activation of the prostatic or the red-cell phosphatases by metals, especially magnesium even after prolonged dialysis, suggests that these acid enzymes do not consist of the metallo-protein structure which has been suggested for the alkaline phosphatases. It might be possible that they are saturated with the metal which is strongly bound to their protein molecule and which cannot be split before the complete denaturation of the protein component of the enzyme. Against this stands the fact that the acid phosphatases are either not affected, or are slightly activated, by cyanides, which strongly and irreversibly inhibit the alkaline enzymes presumably by combination with their essential metal. The inhibition of acid phosphatases by fluorides could not be accounted for by combination of the latter with a metal, since fluorides have either no effect or slightly activate the alkaline phosphatases. The inhibition of acid phosphatases by fluoride can be more reasonably considered as competitive, since Belfanti, Contardi & Ercoli (1935) and Lundquist (1946*a, b*) reported its reversibility by dialysis. The strong inhibitory effect of fluoride on the prostatic enzyme, as compared with its relatively mild action on the red-cell enzyme, is worthy of note.

The high sensitivity of the red-cell acid phosphatases to minute amounts of copper may be taken as evidence for the necessity of thiol groups for the activity of these enzymes. These groups, on the other hand, seem to be not so important for the activity of the prostatic enzyme, since the latter is slightly activated by 0.001M-copper sulphate in citrate buffer. The same explanation can be applied to the

peculiar action of formaldehyde, which under certain conditions completely and irreversibly inhibits the red-cell phosphatases while not affecting the prostatic enzyme. (The action of formaldehyde can also be explained on the basis of the necessity of an amino group, which Gould (1944) claims to be essential for complete activity of the intestinal alkaline phosphatase.) Iodoacetate, however, was found to inhibit both the red-cell and the prostatic acid phosphatases nearly to the same extent. This result is against the hypothesis that thiol groups are not necessary for the activity of the prostatic enzyme, unless this inhibition is due to some other reason than combination with thiol groups. On the other hand, the relative ineffectiveness of this reagent as thiol inhibitor has often been pointed out in the literature, and many well-known thiol enzymes have been found to be unaffected by iodoacetate (Singer, 1948; Hopkins, Morgan & Lutwak-Mann, 1938). The possibility that the thiol groups in the prostatic enzyme are attacked by certain reagents but not by others cannot be overlooked. This point needs more investigation using a variety of effective thiol reagents. The inhibition of sulphhydryl enzymes by heavy metals has been successfully reversed by thiols, e.g. cysteine or glutathione (Hellerman, 1937; Barron & Kalnitsky, 1947). In the present experiments the inhibition of the red-cell phosphatase by copper could not be reversed by thiols. It seems that the copper-mercaptide combination of the red-cell enzyme is so stable, that, unless an appropriate thiol compound is able to dissociate the enzyme-copper complex, the inhibition is irreversible. Neither cysteine nor glutathione seems to have the power to do so.

The importance of the nature of the buffering substance in the study of activators and inhibitors on phosphatases has been pointed out by Lundquist (1947), who found that the hydrolysis of phosphoryl choline and β -glycerophosphate by the prostatic enzyme is much faster in citrate buffer than in acetate buffer (10–30 and 100–500 % respectively). This may be the reason for the very slow hydrolysis of β -glycerophosphate by red cells in acetate buffer; and might furnish a useful clinical method for the determination of prostatic acid phosphatase in serum in methods where β -glycerophosphate is used as substrate. The same author also found that citrate buffer prevents the inhibition of the prostatic enzyme by certain other organic acids, e.g. maleic.

The inhibition of acid phosphatases other than those of normal plasma and red cells by L-tartrate is particularly interesting since it is stereoisomeric in nature and is highly specific. The application of this phenomenon is of considerable diagnostic value in elucidating the slightly raised values for acid phosphatase in the serum in doubtful cases of prostatic cancer. It has been tried in several cases and

the results were very satisfactory. In this respect, the use of L-tartrate is similar to, but is better than, Herbert's (1946) technique of incubation with ethanol. It does not interfere with the corpuscular enzyme, and thus haemolysis causes no error; and the plasma acid phosphatase in non-malignant cases is not affected by L-tartrate. This was established by trying the effect of L-tartrate on plasma acid phosphatase in forty-nine different non-prostatic cases. In about 80 % of these cases the total acid phosphatase was either not affected, or only very slightly affected (± 0.5 unit) by L-tartrate, while when the same plasma were subjected to the ethanol technique only 40 % of them were unaffected. Even in liver disease, where there is a raised total acid phosphatase in most cases, these high values were not significantly affected by L-tartrate.

The liver acid phosphatase was found to be inhibited by L-tartrate, while the bile enzyme was not affected (cf. Table 11). This may be due to certain substances present in the bile that protect the enzyme against such inhibition. Thus the slightly high serum acid phosphatase which occurs during liver disease and which, in many cases, is accompanied by high concentrations of bile constituents, would be expected not to be affected by L-tartrate. This protective action may be due to bile salts which inhibit other acid phosphatases, but have no such effect on the plasma enzyme.

The above facts provide sufficient evidence to conclude that the acid phosphatases of the prostate gland and of the red cell are different enzymes.

Differences amongst the acid phosphatases of other tissues are also demonstrable. For example, the red-cell enzyme is not identical with that of the spleen, as was indicated by Davies (1934). The kidney acid phosphatase also does not seem to be identical with the liver or spleen enzymes, since the former is partially inhibited by ethanol, while the two latter enzymes are not affected. Kochakian & Fox (1944) studied the effect of castration and testosterone propionate on the acid and alkaline phosphatases of the kidney, liver and intestine of the mouse. They found that the activity of the acid phosphatase of kidney was increased with testosterone propionate and decreased by castration while the liver and intestinal enzymes were not affected.

Stilboestrol, together with other oestrogens, is widely used for the treatment of prostatic cancer. As a sequence of favourable response to treatment the plasma acid phosphatase values, which are particularly high in patients with secondaries in the bones, fall markedly during the course of oestrogenic therapy. It was not known whether stilboestrol has a direct inhibitory action on the prostatic and other tissue acid phosphatases, but the present investiga-

tion shows that it has not. The following clinical case illustrates the indirect effect of oestrogens on the prostatic acid phosphatase *in vivo*.

A young man 25 years old was admitted to hospital complaining of pain under the sternum for 3 weeks, cough and dyspnoea for 5 days. He was well built and hair distribution and external genitals were normal. Both breasts were visibly enlarged; there was no other significant physical sign. The Aschheim-Zondek test was positive with a urine dilution of 1:10, and a serum dilution 1:100, giving a concentration of 4000 to 40,000 and between 40,000 and 400,000 mouse units of gonadotrophic hormone/l. The blood count was significantly abnormal. He was regarded as a case of generalized chorion-epithelioma, primary unknown, there being no palpable lesion of the testes. The patient deteriorated rapidly and died. The summary of lesions found on post-mortem examination is as follows: (1) chorion-epithelioma arising in superior mediastinum presumably in the teratoma; (2) massive pulmonary metastases; (3) gynaeomastia. There was one metastasis, 9 mm. diameter in the inferior part of the left lobe of the cerebellum; the brain was otherwise normal.

The two breasts were dissected out and weighed 95 g. Macroscopically they resembled a non-lactating female breast. They were sharply defined from the surrounding fat. They were examined for alkaline phosphatase and were found to be very poor in this enzyme.

The thyroid, parathyroid and pituitary were normal. The cortex of the suprarenal appeared somewhat thinned. Both testes were of normal size and quite free from tumour or any abnormality. The seminal vesicles appeared enlarged. The prostate gland appeared quite normal in size. It was examined for phosphatase activity, and it was found that 1 g. wet weight of prostate liberated 126 mg. phenol from phenyl phosphate at pH 5, during 1 hr. at 37°. The normal human prostate gland liberates 3000–10,000 mg. phenol/g. wet weight under the same conditions.

It is, therefore, believed that the gonadotrophic hormones, through their indirect action on the prostate cells, cause less phosphatase to be produced. A parallel action is believed to take place when oestrogens are administered in the treatment of cancer of the prostate with secondaries.

SUMMARY

1. The prostatic and red-cell acid phosphatases have been studied with respect to pH-hydrolysis curves of phenyl phosphate, the relative rates of hydrolysis of α - and β -glycerophosphates, and the effect of magnesium. The presence of two acid phosphatases in the red cells has been confirmed.

2. The effects of calcium, chromium, cobalt, copper, iron, manganese, nickel and zinc on the activity of the prostatic and red-cell enzymes are described. No evidence for enzyme activation by any of these metals was obtained. Copper completely destroys the red-cell enzyme; while iron, in acetate buffer, strongly inhibits the prostatic enzyme, but has no such action in citrate buffer.

3. Glycine and alanine have no effect on either prostatic or red-cell phosphatases. Cysteine slightly activates both enzymes.

4. The effects of ethanol, acetone, formaldehyde and certain organic acid radicals on acid phosphatase activity have been studied. L-Tartrate,

while not affecting the acid phosphatases of plasma and red cells, inhibits other acid phosphatases particularly that of the prostate, and the nature of this action has been studied and discussed.

5. Stilboestrol has no direct effect on any of the acid phosphatases.

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The Micro- and Ultramicro-determination of Chlorine in Organic Compounds

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To follow the metabolism of 1:1:1-trichloro-2:2-di-(p-chlorophenyl) ethane (DDT) in tissues a method was necessary for determining small amounts of organically bound chlorine. In addition, a technique was required for the estimation of DDT in the presence of possible degradation products. Although the determination of inorganic chloride in minute amounts is possible (Sendroy, 1937, 1939; Conway, 1935), adaptation to the new requirements would not be convenient since in Sendroy's method chloride is precipitated with silver iodate and the concentration of chloride must be at least 0.01 M. The method of Conway is not subject to these limitations, but the dimensions of his microdiffusion unit are such that it would be difficult to make estimations of small amounts of organically bound chlorine in relatively large amounts of tissue.

The basis of the present method is the photometric determination of the intensity of the starch-iodine blue colour after liberation of free iodine from potassium iodide by chlorine formed by the oxidation of the compound under test.

A technique has also been devised for the determination of DDT in tissue extracts in the presence of metabolites. The discovery by White & Sweeney (1945) that 2:2-di(p-chlorophenyl)acetic acid (DDA) is a product of DDT metabolism in animals has made it possible to separate an alkali-soluble from a neutral fraction of organically bound chlorine found in the tissues of animals after the ingestion of DDT.

METHODS AND RESULTS

Oxidation of organically bound chlorine is effected by heating with specially prepared silver dichromate in the presence of concentrated sulphuric acid. Inorganic chloride may be determined after its oxidation in the cold by an acid permanganate mixture (Conway, 1935), organically bound chlorine not being attacked under these conditions. The latter method may be used for the specific determination of DDT since 1 mol. of hydrochloric acid is liberated/mol. of DDT by the action of alcoholic alkali (Gunther, 1945). Chlorine volatilized by the oxidation is carried