Heterogeneity of Liver Acid Phosphatases in Developing Chick Embryo

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1. The development, localization and heterogeneity of acid phosphatase and a Zn^{2+} -activated acid phosphatase in cellular fractions of developing chick liver were studied. 2. Acid phosphatase is distributed abundantly in the particulate and soluble fractions. The soluble fraction is rich in Zn^{2+} -activated acid phosphatase, which attains its peak activity at about 15 days of incubation. 3. The particulate acid phosphatase activity is inhibited by fluoride but not by sodium L(+)-tartrate or cysteine. On the other hand, the soluble Zn^{2+} -activated acid phosphatase activity is inhibited by sodium L(+)-tartrate and cysteine but not by fluoride. 4. The pH optimum of these two enzymes is similar at about 5.6. 5. The soluble Zn^{2+} -activated acid phosphatase activity appears to be thermally stabilized by the treatment with Triton X-100 or bovine serum albumin.

Acid phosphatases, which hydrolyse the phosphomonoesters of alcohols and phenols, are exceptionally abundant in liver, kidney, erythrocytes and prostate gland. The presence of these phosphatases in various organs of the developing chick embryo has been reported by many investigators (Russo-Caia, 1962; Moog, 1965; Salzgeber & Weber, 1966; Wang, 1968). Heterogeneity of acid phosphatase activities has been shown in the soluble fractions of liver and brain from several species (Moore & Angeletti, 1961), in rat and guinea-pig liver extracts (Nelson, 1966; Neil & Horner, 1964a), in human diploid cell strain WI-38 (Cristofalo, Kabakjian & Kritchevsky, 1967), in human erythrocytes (Scott, 1966; Fenton & Richardson, 1967) and in leucocytes (Yu, Kummerow & Nishida, 1966). Acid phosphatase has generally been recognized as one of the lysosomal enzymes, which can be released by the treatment with Triton X-100 (de Duve, Pressman, Gianetto, Wattiaux & Appelmans, 1955). A *p*-nitrophenyl acid phosphatase has been found in the liver microsomal fraction of guinea-pig and mouse liver (Neil & Horner, 1964a,b). This enzyme has little action on β -glycerophosphate and differs from the supernatant enzyme of guinea-pig liver extracts which is inhibited to a smaller degree by fluoride. The effects of fluoride on lysosomal and supernatant enzymes in rat liver, WI-38 human diploid cells and leucocytes are similar to those on guinea-pig liver microsomal and supernatant enzymes. These findings suggest that the supernatant acid phosphatase from various

tissues of different species is similar with respect to its inhibition by fluoride but differs from the acid phosphatase from lysosomes or microsomes. Histochemical studies of alkaline and acid phosphatase activities (Moog, 1965) in relation to differentiation of a specific tissue have been reported, but the characteristics of these phosphatases from various cellular compartments during development and differentiation are not known. The question thus arises as to whether different phosphatase activities are also characteristic of various cellular compartments in a differentiated tissue. The present paper describes the study on the localization of these phosphatases: acid phosphatase (EC 3.1.3.2) and a Zn^{2+} -activated acid phosphatase in developing chick liver. Results on the heterogeneity of these phosphatases in various cellular fractions during embryonic development are also presented.

MATERIALS AND METHODS

Fertilized Leghorn eggs purchased from a local hatchery were incubated and used throughout all the experiments. The temperature, humidity and method of dissection of tissues have been described previously (Wang, 1966, 1967).

Liver fractions. Chicken livers were obtained at various developmental stages. A 10% (w/v) homogenate was made with cold 0.25 M-sucrose solution in a Potter-Elvehjem homogenizer having a clearance of 0.01–0.15 mm. The Teflon pestle of the homogenizer was rotated at 12000 rev./min. and the homogenization was completed by ten up-and-down strokes. Two procedures of differential centrifugation were carried out as follows: (a) The homogenate (H) was

centrifuged at 700g for 10 min. to give a nuclear fraction (N), which was washed once with 0.25M-sucrose. The combined supernatant (I) was centrifuged at 15000g for 10 min. to obtain the mitochondrial fraction (M). This was washed once and the combined supernatant (II) centrifuged at 105000g for 1 hr. to give a particulate fraction (P) and a soluble fraction (S). (b) In some experiments the lysosomal fraction was obtained and analysed. This procedure was similar to (a) except that the combined supernatant (I) was centrifuged at 5000g for 20 min. to obtain the mitochondrial fraction (M). This was washed once and the combined supernatant (II) centrifuged at 16500g for 20 min. to obtain the lysosomal fraction (L).

Chemicals. Versatol (freeze-dried human serum) was purchased from Warner-Chilcott, Morris Plains, N.J., U.S.A. *p*-Nitrophenyl phosphate disodium salt, *p*-nitrophenol, L-cysteine and bovine serum albumin were purchased from Sigma Chemical Co., St Louis, Mo., U.S.A. Triton X-100 was purchased from Rohm and Hass Co., Philadelphia, Pa., U.S.A. All the other chemicals were purchased from Fisher Scientific Co., Pittsburg, Pa., U.S.A.

Enzyme assays. Phosphatase activities of the cellular fractions were determined by the method of Lowry, Roberts, Wu, Hixon & Crawford (1954). In the assays for Zn^{2+} -activated acid phosphatase (Wang, 1968) and acid phosphatase activities, the samples (in a final volume of 120 μl .) were incubated for 30 min. at 37° with 50 mM-sodium acetate buffer, pH 5.6 or 5.2, in the presence or absence of 5 mM- ZnSO_4 and *p*-nitrophenyl phosphate (8 mM). The reaction was stopped by the addition of 400 μl . of 0.15M-NaOH and the extinction of the liberated *p*-nitrophenol read at 410 nm. Solvent blanks, standards (*p*-nitrophenol) and sample blanks were carried out simultaneously. The phosphatase activities are expressed as μmoles of *p*-nitrophenol liberated/hr./mg. of protein. All the enzymic assays were performed under the above conditions unless otherwise specified. The

protein content of various cellular fractions was determined by the method of Lowry, Rosebrough, Farr & Randall (1951) with Versatol as standard. All buffers, substrates and other reagents were made with demineralized double-glass-distilled water.

RESULTS

Table 1 (*a* and *b*) shows the acid phosphatase and Zn^{2+} -activated acid phosphatase activities and their distribution in various cellular fractions prepared from 1-day-old chick liver by procedures (*a*) and (*b*). The results show that their activities in the particulate and the soluble fractions as well as their distributions and recoveries obtained by these two procedures were essentially the same. Since the contribution by the lysosomal fraction of acid phosphatase and Zn^{2+} -activated acid phosphatase activities was small, the first procedure of differential centrifugation, which provides no separation of this fraction, was used throughout all the following experiments.

The emphasis of the present paper is primarily on the acid phosphatase and Zn^{2+} -activated acid phosphatase activities from the particulate and the soluble fractions. The cellular fractions were prepared without Triton X-100, unless otherwise specified.

Development of phosphatases in cellular compartments of chick liver. As shown in Table 2 the overall acid phosphatase activity in the whole liver homogenate remained practically constant during the developmental period. However, there were

Table 1. *Acid phosphatase activities in cellular fractions of 1-day-old chick liver*

Details of the assays used for each enzyme by procedure (*a*) or (*b*) are given in the Materials and Methods section. Acid phosphatase and Zn^{2+} -activated acid phosphatase activities are expressed as μmoles of *p*-nitrophenol liberated/hr./mg. of protein.

Cellular fraction	Acid phosphatase		Zn^{2+} -activated acid phosphatase	
	Activity	Distribution (%)	Activity	Distribution (%)
Procedure (<i>a</i>)				
Nuclear ($7 \times 10^3 \text{g-min.}$)	1.38	4	5.52	3
Mitochondrial ($1.5 \times 10^5 \text{g-min.}$)	2.34	6	2.34	2
Particulate ($6.3 \times 10^6 \text{g-min.}$)	7.18	45	4.09	6
Soluble	3.12	38	29.55	83
Whole homogenate	3.17		15.79	
Recovery		93		94
Procedure (<i>b</i>)				
Nuclear ($7 \times 10^3 \text{g-min.}$)	1.84	4	4.10	3
Mitochondrial (10^5g-min.)	2.00	3	2.93	1
Lysosomal ($3.3 \times 10^5 \text{g-min.}$)	2.95	3	3.08	1
Particulate ($6.3 \times 10^6 \text{g-min.}$)	6.92	44	4.11	8
Soluble	2.98	39	28.28	82
Whole homogenate	3.47		14.24	
Recovery		93		95

Table 2. *Acid phosphatase activities in cellular fractions of developing chick liver*

Procedures for separation of the fractions and enzymic assay are described in the Materials and Methods section. Acid phosphatase activity is expressed as μ moles of *p*-nitrophenol liberated/hr./mg. of protein. Each value is the average $\pm$ S.E.M. of four experiments; each experiment from each incubation day contains five to 20 embryos or two to three chicks.

Incubation (days)	Acid phosphatase activity			
	H	M	P	S
8	4.71 $\pm$ 0.18	5.38 $\pm$ 0.27	4.39 $\pm$ 0.27	3.39 $\pm$ 0.49
10	4.83 $\pm$ 0.17	4.97 $\pm$ 0.54	7.01 $\pm$ 0.06	3.39 $\pm$ 0.35
13	4.33 $\pm$ 0.02	3.70 $\pm$ 0.17	7.09 $\pm$ 0.43	4.20 $\pm$ 0.34
15	4.48 $\pm$ 0.48	3.58 $\pm$ 0.43	7.77 $\pm$ 0.49	4.01 $\pm$ 0.95
17	4.55 $\pm$ 0.23	3.48 $\pm$ 0.45	6.52 $\pm$ 0.37	4.09 $\pm$ 0.39
20	3.91 $\pm$ 0.23	2.16 $\pm$ 0.27	7.50 $\pm$ 0.48	3.48 $\pm$ 0.32
1-day-old chick	3.83 $\pm$ 0.33	2.21 $\pm$ 0.10	7.64 $\pm$ 1.14	3.29 $\pm$ 0.20
8-day-old chick	4.87 $\pm$ 0.16	3.33 $\pm$ 0.23	8.88 $\pm$ 0.47	2.78 $\pm$ 0.49

Table 3. *Zn²⁺-activated acid phosphatase activities in cellular fractions of developing chick liver*

Procedures for separation of the fractions and enzymic assay are described in Materials and Methods section. Zn²⁺-activated acid phosphatase activity is expressed as μ moles of *p*-nitrophenol liberated/hr./mg. of protein. Each value is the average $\pm$ S.E.M. of four experiments; each experiment from each incubation day contains five to 20 embryos or two to three chicks.

Incuba- tion (days)	Zn ²⁺ -activated acid phosphatase activity			
	H	M	P	S
8	11.71 $\pm$ 0.40	2.76 $\pm$ 0.48	3.50 $\pm$ 0.44	20.08 $\pm$ 2.12
10	17.02 $\pm$ 1.11	3.42 $\pm$ 0.28	4.56 $\pm$ 0.30	34.63 $\pm$ 1.89
13	19.24 $\pm$ 0.54	2.88 $\pm$ 0.08	4.49 $\pm$ 0.32	39.27 $\pm$ 1.49
15	18.89 $\pm$ 1.85	2.65 $\pm$ 0.34	3.57 $\pm$ 0.32	39.36 $\pm$ 3.06
17	16.56 $\pm$ 1.84	2.59 $\pm$ 0.29	3.57 $\pm$ 0.13	38.56 $\pm$ 6.52
20	15.91 $\pm$ 2.07	2.65 $\pm$ 0.40	4.00 $\pm$ 0.32	36.15 $\pm$ 9.97
1-day-old chick	14.05 $\pm$ 0.89	2.01 $\pm$ 0.17	3.89 $\pm$ 0.32	28.38 $\pm$ 0.61
8-day-old chick	10.74 $\pm$ 0.88	2.52 $\pm$ 0.04	3.53 $\pm$ 0.21	20.21 $\pm$ 1.62

some minor fluctuations of this enzymic activity in the mitochondrial and particulate fractions during the first week of development. By comparison, the Zn²⁺-activated acid phosphatase activity was highest in the soluble fraction and reached a peak between 15–17 days of incubation, whereas little change in enzymic activity occurred in the mitochondrial and the particulate fractions (Table 3). These results show clearly the differences in

Table 4. *Effect of EDTA on soluble Zn²⁺-activated acid phosphatase activity of 15-day-old embryonic liver*

Details of the assay used for Zn²⁺-activated acid phosphatase are given in the Materials and Methods section. Enzyme activity is expressed as μ moles of *p*-nitrophenol liberated/hr./mg. of protein.

Concn. of EDTA (mm)	Concn. of Zn ²⁺ (mm)	Zn ²⁺ -activated acid phosphatase	
		Activity	% of control activity
0	5	34.12	100
0	0	2.21	6
1	0	2.89	8
5	0	2.86	8
1	5	26.65	78
1	10	27.88	82
1	50	13.75	40

Fig. 1. Inhibition of acid phosphatase (a) and Zn²⁺-activated acid phosphatase (b) activities in liver particulate (P) and soluble (S) fractions. □, Liver cellular fraction of 8-day-old embryo; ▨, liver cellular fraction of 1-day-old chick. All the inhibitors were added to give a final concentration of 50 mm.

development and location of these two acid phosphatases in developing chick liver.

Treatment with EDTA. To assess the nature of the residual activity of the soluble Zn²⁺-activated acid phosphatase in the absence of Zn²⁺, EDTA was added to the assay system. The results of such a study are shown in Table 4. The acid phosphatase

activity in the soluble fraction of 15-day-old embryonic liver was 6% and, in the presence of 1mM- or 5mM-EDTA, 8% of that assayed in the presence of Zn^{2+} . The residual enzymic activity was therefore less than 10% of the total Zn^{2+} -activated acid phosphatase activity and was probably not due to intrinsic Zn^{2+} . That Zn^{2+} serves as an activator of the enzyme is shown by the restoration of enzyme activity on reintroducing Zn^{2+} to the EDTA-treated assay system.

Inhibition of acid phosphatases. The effects of tartrate, fluoride, cyanide and cysteine, at 50mM, on the particulate and soluble acid phosphatase and Zn^{2+} -activated acid phosphatase activities of the 8-day-old and 15-day-old embryonic livers and of 1-day-old chick liver were studied (Fig. 1). The results indicate that the two enzyme activities in the particulate fraction of chick embryonic livers responded to the inhibitors differently from the soluble enzymes. Although cyanide completely inhibited both phosphatases, soluble and particulate, the strong inhibition of the soluble Zn^{2+} -activated acid phosphatase activity by cysteine and tartrate is evidently specific. These characteristics are apparently related to their cellular origin. Some

quantitative differences in the inhibition of the two acid phosphatases are also noticeable in the three developmental stages studied.

To explore further the response of the soluble Zn^{2+} -activated acid phosphatase and particulate acid phosphatase towards the inhibitors, cyanide and tartrate were tested at two different concentrations, namely 10mM and 50mM. The results of such a study are summarized in Table 5. Cyanide at 10mM had practically no effect on the Zn^{2+} -activated acid phosphatase activity, but at 50mM caused a complete cessation of both phosphatase activities. Sodium L(+)-tartrate at either 10 or 50mM inhibited Zn^{2+} -activated acid phosphatase activity, but had no effect at 50mM and was only slightly inhibitory at 10mM on acid phosphatase activity. Since the effect of sodium L(+)-tartrate on particulate acid phosphatase activities appears to differ from the commonly known inhibition by L(+)-tartaric acid, a further examination of the inhibitory effect of L(+)-tartaric acid was made (Table 5). Indeed, L(+)-tartaric acid inhibited the particulate acid phosphatase and the soluble Zn^{2+} -activated acid phosphatase activities.

The Zn^{2+} -activated acid phosphatase, which requires Zn^{2+} for maximal activity, is inhibited by cysteine. Experiments were designed to test the supposition that the inhibition might be due to competition for a binding site between the cysteine and bivalent ions. When Zn^{2+} and cysteine were added at zero time or when cysteine was added and incubated 15 min. before the addition of Zn^{2+} , the Zn^{2+} -activated acid phosphatase activities were similar (85 and 82% inhibition; Table 6). However, a 40% inhibition resulted when Zn^{2+} was

Table 5. *Effect of inhibitors on chick liver phosphatases*

Inhibitor	Concn. (mM)	Incubation (days)	Inhibition (%)	
			Soluble Zn^{2+} -activated acid phosphatase activity	Particulate acid phosphatase activity
Cyanide	10	8	-3*	74
		15	-10*	82
		1-day-old chick	-11*	65
	50	8	100	99
		15	100	99
		1-day-old chick	100	98
Sodium L(+)-tartrate	10	8	66	19
		15	66	18
		1-day-old chick	66	13
	50	8	87	-4*
		15	91	-7*
		1-day-old chick	92	-12*
L(+)-Tartaric acid	50	15	90	75
	50	1-day-old chick	98	81

* Minus sign indicates activation.

Table 6. *Reaction of 15-day-old embryonic liver soluble Zn^{2+} -activated acid phosphatase with inhibitors*

Zn^{2+} -activated acid phosphatase activity is expressed as μ moles of *p*-nitrophenol liberated/hr./mg. of protein. S, Liver soluble fraction. Zn^{2+} , $ZnSO_4$ at a final concentration of 5mM; Cys, L-cysteine at a final concentration of 50mM.

Conditions	Zn^{2+} -activated acid phosphatase	
	Activity	% of control activity
S	2.66	7
S + Zn^{2+} (control)	36.34	100
S + Cys	2.80	8
S + Zn^{2+} + Cys	5.61	15
S + Cys (incubated 15 min.) + Zn^{2+}	6.64	18
S + Zn^{2+} (incubated 15 min.) + Cys	21.99	60

Fig. 2. Effect of pH on acid phosphatase activity in liver homogenate (●), particulate (○) and soluble (△) fractions of 15-day-old chick embryo. The pH was adjusted to values 4.0–6.0 with 50 mM-acetate buffer, and to values 6.5–7.5 with 50 mM-acetate–50 mM-cacodylate buffer. Assays were carried out at 37° with 8 mM-*p*-nitrophenyl phosphate for 30 min. Each point was an average of two experiments. The maximum activity was expressed by taking the highest activity in any fraction as 100% and the other activities were expressed as a percentage of the maximum activity.

Fig. 3. Effect of pH on Zn^{2+} -activated acid phosphatase activity in liver homogenate (●), particulate (○) and soluble (△) fractions of 15-day-old chick embryo. The pH was adjusted to values 4.0–6.0 with 50 mM-acetate buffer, and to values 6.5–7.5 with 50 mM-acetate–50 mM-cacodylate buffer. Assays were carried out at 37° with 8 mM-*p*-nitrophenyl phosphate and 5 mM- $ZnSO_4$ for 30 min. Each point was an average of two experiments. The maximum activity was expressed by taking the highest activity in any fraction as 100% and the other activities were expressed as a percentage of the maximum activity.

added and incubated before the addition of cysteine. These findings thus suggest that in the Zn^{2+} -activated acid phosphatase system cysteine may compete with Zn^{2+} or may bind with Zn^{2+} resulting in partial inhibition of the enzymic activity.

Heterogeneity of acid phosphatase. To examine the heterogeneity of phosphatase during development, experiments were conducted to test possible differences of pH optima of acid phosphatase and Zn^{2+} -activated acid phosphatase activities from

Fig. 4. Effect of pH and metal ions on acid phosphatase activity in liver particulate (a) and soluble (b) fractions of 1-day-old chick. All the metal ions were added to give a final concentration of 5 mM. The pH was adjusted to values 4.0–6.0 with 50 mM-acetate buffers, and to 6.5 with 50 mM-acetate–50 mM-cacodylate buffers. Assays were carried out at 37° with 8 mM-*p*-nitrophenyl phosphate and with or without metal ions. ○, Mn^{2+} ; △, Mg^{2+} ; □, Zn^{2+} ; ●, no metal ions added.

cellular fractions of 15-day-old embryonic liver. Acetate buffers were used for the pH range 4.0–6.0, and acetate–cacodylate buffers for the pH range 6.5–7.5. As shown in Figs. 2 and 3, two main activity peaks, at pH 5.2 and at pH 5.6–5.8, were observed for the acid phosphatase in the homogenate, particulate and soluble fractions, and at pH 5.6–6.0 for Zn^{2+} -activated acid phosphatase. A minor activity peak at pH 5.2 may be present in the Zn^{2+} -activated acid phosphatase. These results suggest the multiplicity of the two acid phosphatases of chick embryonic liver.

Since pH studies could not distinguish qualitatively between the acid phosphatase and Zn^{2+} -activated acid phosphatase of the particulate and the soluble fractions, experiments were performed to study the effect of ions on acid phosphatase activity from the soluble and particulate fractions of 1-day-old chick liver in an attempt to reveal any possible qualitative differences. As shown in Fig. 4, bivalent cations did not shift the pH optimum of the particulate acid phosphatase. The particulate acid

Fig. 5. Thermal stability of particulate and soluble acid phosphatase and Zn^{2+} -activated acid phosphatase activities at 50° in fractions from the liver of 1-day-old chick. (a), (b) and (c), Acid phosphatase; (d), (e) and (f), Zn^{2+} -activated acid phosphatase. ●, Particulate fraction; ○, soluble fraction. In (a) and (d) the samples were diluted with appropriate amounts of 50 mM-acetate buffer, pH 5.2 or 5.6. In (b) and (e) the samples were diluted similarly to those in (a) and (d) but were also made 1% (v/v) with respect to Triton X-100 and left for 30 min. before assay. The samples in (c) and (f) were diluted with appropriate amounts of 0.05% (w/v) bovine serum albumin. Zn^{2+} -activated acid phosphatase (with 5 mM- ZnSO_4 at pH 5.6) and acid phosphatase (at pH 5.2) activities were assayed in 50 mM-acetate buffer and 8 mM-*p*-nitrophenyl phosphate.

phosphatase activity was increased more with Mn^{2+} than with the other bivalent cations tested. Although Zn^{2+} had no activating effect on the particulate acid phosphatase, it gave the greatest activation of the soluble acid phosphatase activity. Mn^{2+} could substitute for Zn^{2+} but gave only 40% activation, whereas Mg^{2+} showed no effect.

Thermal stability. The thermal stability of acid phosphatase and Zn^{2+} -activated acid phosphatase from the liver particulate and the soluble fractions was examined after various times of incubation at 50° . Acid phosphatase samples from the particulate and the soluble fractions were diluted and mixed for 30 min. with appropriate amounts of 0.05% bovine albumin, or with 50 mM-sodium acetate buffer, pH 5.2, with or without the addition of Triton X-100 (1%, v/v), then assayed. Zn^{2+} -activated acid phosphatase samples from the above two fractions were diluted similarly except that the acetate buffer used was pH 5.6. The results are shown in Fig. 5. Under all three conditions, the soluble acid phosphatase lost its activity faster than the particulate enzyme (Figs. 5a, 5b and 5c). The thermal denaturation patterns may also suggest the presence of a second enzyme. However, Triton and bovine serum albumin treatment apparently protected the soluble Zn^{2+} -activated

acid phosphatase from decay to some extent (Figs. 5e and 5f). It is noteworthy that bovine serum albumin increased the rate of decay of both particulate enzyme activities. In general, these results suggest differences in thermal stabilities between the acid phosphatase and Zn^{2+} -activated acid phosphatase on the one hand, and between the soluble and particulate enzymes on the other.

DISCUSSION

Some loss of activity of both acid phosphatases occurred during fractionation (Table 1). The loss of acid phosphatase activity seems to be due to the oxidation of thiol groups, since the effect of cysteine on this enzymic activity results in slight activation. In comparison, Zn^{2+} -activated acid phosphatase activity (soluble) is not dependent on the reduced thiol groups but rather dependent on bivalent cations (Wang, 1968).

The present study shows an apparent shift of acid phosphatase activity from the mitochondrial fraction to the particulate fraction during liver development. Solomon (1959) has observed a shift of glutamate dehydrogenase activity from the chick liver soluble fraction to the mitochondrial fraction during the last quarter of incubation. Whether or

not these shifts reflect changes in cellular processes is unknown. No apparent shift of Zn^{2+} -activated acid phosphatase activity from one cellular fraction to another was observed. The maximal increase of liver soluble Zn^{2+} -activated acid phosphatase on about day 15 of incubation may be related to the liver functional development, since chick embryonic liver starts to function about 2 weeks after incubation (Dalton, 1937; Lee, 1951; O'Connor, 1953).

The results of the studies with inhibitors, of pH effect and of temperature on acid phosphatase and Zn^{2+} -activated acid phosphatase activities demonstrate the heterogeneities of these acid phosphatases. The characteristics of fluoride inhibition, the reaction with cysteine and the pH optimum at 5.2 of the particulate acid phosphatase are the same as those reported for acid phosphatase from guinea-pig liver (Neil & Horner, 1964a), rat liver (Nelson, 1966), WI-38 diploid cells (Cristofalo *et al.* 1967) and leucocytes (Yu *et al.* 1966). Whether these similarities indicate that the same acid phosphatase species are present in various tissues is not known.

The pH optimum, thermal stability and the response to tartrate, fluoride, cyanide and cysteine of the particulate Zn^{2+} -activated acid phosphatase are similar to those of the particulate acid phosphatase. These plus the greater activation of the liver particulate acid phosphatase activity by Mn^{2+} and Mg^{2+} , but not by Zn^{2+} , suggest that the existence of particulate Zn^{2+} -activated acid phosphatase is doubtful.

That the soluble Zn^{2+} -activated acid phosphatase is another enzyme species is indicated by its reaction with sodium L(+)-tartrate, fluoride and cysteine, by its activation by Zn^{2+} and Mn^{2+} and by its stability when treated with Triton X-100 and bovine serum albumin. The stabilizing mechanism of the soluble Zn^{2+} -activated acid phosphatase is not clear. Bovine serum albumin and Triton X-100 may act similarly as surface-active substances. The stabilization of soluble Zn^{2+} -activated acid phosphatase activity with Triton X-100 and bovine serum albumin may be explained similarly as the result observed by Dowben & Koehler (1961) and Koehler & Dowben (1961), i.e. the degree of

hydration is increased in the presence of Triton X-100. Thus the enzyme is insulated from the surrounding environment and rendered less susceptible to denaturation. Other factors, however, might have been involved in this stabilization phenomenon.

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REFERENCES

- Cristofalo, V. J., Kabakjian, J. R. & Kritchevsky, D. (1967). *Proc. Soc. exp. Biol., N. Y.*, **126**, 649.
 Dalton, A. J. (1937). *Anat. Rec.* **68**, 393.
 de Duve, C., Pressman, B. C., Gianetto, R., Wattiaux, R. & Appelmans, F. (1955). *Biochem. J.* **60**, 604.
 Dowben, R. M. & Koehler, W. R. (1961). *Arch. Biochem. Biophys.* **93**, 496.
 Fenton, M. R. & Richardson, K. E. (1967). *Arch. Biochem. Biophys.* **120**, 332.
 Koehler, W. R. & Dowben, R. M. (1961). *Arch. Biochem. Biophys.* **93**, 501.
 Lee, W. H. (1951). *Anat. Rec.* **110**, 465.
 Lowry, O. H., Roberts, N. R., Wu, M. L., Hixon, W. S. & Crawford, E. J. (1954). *J. biol. Chem.* **207**, 19.
 Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). *J. biol. Chem.* **193**, 265.
 Moog, F. (1965). In *The Biochemistry of Animal Development*, vol. 1, pp. 314-334. Ed. by Weber, R. New York: Academic Press Inc.
 Moore, B. W. & Angeletti, P. U. (1961). *Ann. N.Y. Acad. Sci.* **94**, 659.
 Neil, M. W. & Horner, M. W. (1964a). *Biochem. J.* **92**, 217.
 Neil, M. W. & Horner, M. W. (1964b). *Biochem. J.* **93**, 220.
 Nelson, B. D. (1966). *Proc. Soc. exp. Biol., N. Y.*, **121**, 998.
 O'Connor, R. J. (1953). *J. Embryol. exp. Morph.* **1**, 105.
 Russo-Caia, S. (1962). *Acta Embryol. Morph. exp.* **5**, 1.
 Salzgeber, P. B. & Weber, R. (1966). *J. Embryol. exp. Morph.* **15**, 397.
 Scott, E. M. (1966). *J. biol. Chem.* **241**, 3049.
 Solomon, J. B. (1959). *Developmental Biol.* **1**, 182.
 Wang, K.-M. (1966). *Life Sci.* **5**, 2209.
 Wang, K.-M. (1967). *Europ. J. Pharmacol.* **1**, 347.
 Wang, K.-M. (1968). *Experientia*, **24**, 424.
 Yu, B. P., Kummerow, F. A. & Nishida, T. (1966). *Proc. Soc. exp. Biol., N. Y.*, **122**, 1045.