mum amounts of magnesium chloride in the digest. The effect of activators on the relationship between pH and K_m is discussed.

The author is most grateful to Dr A. M. Wynne and Dr H. D. Branion for their advice and encouragement in this work, and to Miss M. Briggs, Miss G. Ritcey and Mr D. Smith for valuable technical assistance.

REFERENCES

Bailey, K. & Webb, E. C. (1944). *Biochem. J.* **38**, 394.
Bodansky, O. (1946). *J. biol. Chem.* **165**, 605.
Briggs, G. E. & Haldane, J. B. S. (1925). *Biochem. J.* **19**, 338.
Dixon, M. (1953). *Biochem. J.* **55**, 161.
Folley, S. J. & Kay, H. D. (1935). *Biochem. J.* **29**, 1837.
Friedenwald, J. S. & Maengwyn-Davies, G. D. (1954). *The Mechanism of Enzyme Action*, p. 154. Ed. by McElroy, W. D. & Glass, B. Baltimore: The Johns Hopkins Press.
Gomori, G. (1949). *J. Lab. clin. Med.* **34**, 275.
Gryder, R. M., Friedenwald, J. S. & Carlson, C. (1955). *Arch. Biochem. Biophys.* **54**, 281.
Hofstee, B. H. J. (1955). *Arch. Biochem. Biophys.* **59**, 398.
Jacobsen, E. (1933). *Biochem. Z.* **267**, 89.
Kaplan, A. & Narahara, A. (1953). *J. Lab. clin. Med.* **41**, 819.
Kutscher, W. & Wörner, A. (1936). *Z. phys. Chem.* **239**, 109.
Laidler, K. J. (1955). *Trans. Faraday Soc.* **51**, 528.
Lineweaver, H. & Burk, D. (1934). *J. Amer. chem. Soc.* **56**, 658.
Michaelis, L. & Menten, M. L. (1913). *Biochem. Z.* **49**, 333.
Morton, R. K. (1952). Ph.D. Thesis, University of Cambridge.
Morton, R. K. (1957). *Biochem. J.* **65**, 674.
Motzok, I. (1945). Ph.D. Thesis, University of Toronto.
Motzok, I. (1950). *Biochem. J.* **47**, 193.
Motzok, I. & Branion, H. D. (1959). *Biochem. J.* **72**, 177.
Motzok, I. & Wynne, A. M. (1950). *Biochem. J.* **47**, 187.
Neilands, J. B. & Stumpf, P. K. (1955). *Outlines of Enzyme Chemistry*, p. 77. New York: J. Wiley and Sons Inc.
Pfankuch, E. (1936). *Z. phys. Chem.* **241**, 34.
Ross, M. H., Ely, J. O. & Archer, J. G. (1951). *J. biol. Chem.* **192**, 561.
Schønheyder, F. (1951). *Biochem. J.* **50**, 378.
Treloar, A. E. (1939). *Elements of Statistical Reasoning*, p. 108. New York: John Wiley and Sons Inc.
Trubowitz, S., Feldman, D., Benante, C. & Kirman, D. (1957). *Proc. Soc. exp. Biol., N.Y.*, **95**, 35.
Tsuboi, K. K., Wiener, G. & Hudson, P. B. (1957). *J. biol. Chem.* **224**, 621.
Van Slyke, D. D. (1942). *Advanc. Enzymol.* **2**, 33.

Studies on Alkaline Phosphatases

2. FACTORS INFLUENCING pH OPTIMA AND MICHAELIS CONSTANT

By I. MOTZOK and H. D. BRANION
Department of Nutrition, Ontario Agricultural College, Guelph, Canada

(*Received* 22 *May* 1958)

It has become quite evident that the pH required for optimum activity of alkaline phosphatases, as well as for some other enzymes, is not a constant entity. For example, the optimum pH for phosphatases from various sources has been shown to vary with the initial concentration of substrate (Motzok & Wynne, 1950; Ross, Ely & Archer, 1951; Morton, 1957). This phenomenon has been observed with other enzymes, namely urease (Van Slyke & Cullen, 1914) and β-glucosidase (Hofstee, 1955). Other factors, such as buffers (Aebi & Abelin, 1948; Zittle & Della Monica, 1950), type of substrate (Delory & King, 1943; Walker & King, 1950), source of enzyme (Zittle & Della Monica, 1950; Motzok, 1950; Morton, 1955) and activators (Motzok, 1945; Aebi & Abelin, 1948; Sadasivan, 1952; Morton, 1957) also have been found to exert an influence on the optimum pH for phosphatase activity. In our studies on the kinetics of alkaline phosphatases of tissues and plasmas of different species of birds and mammals, the pH optima were determined for a wide range of concentration of substrate with each enzyme preparation. Several factors were found to influence the optimum pH and some of the data are recorded in this paper.

It was demonstrated (Motzok, 1959) that K_m values (Michaelis constant) for chick plasma phosphatase varied with the amount of enzyme in the reaction mixture when the activities were measured at a constant high pH for all dilutions of substrate and enzyme. However, when the activities were measured at the optimum pH for each concentration of substrate, the variation in K_m values due to variation in the concentration of enzyme was largely eliminated. In addition, the plotting of reciprocals of velocities and substrate concentrations, according to Lineweaver & Burk (1934), resolved the data into two parts from which two values for K_m were calculated, K_{m_1} from the data with low concentration of substrate and K_{m_2} from

velocities with high concentrations of substrate. In the present study values for K_{m_1} and K_{m_2}, derived from velocities at the optimum pH for each concentration of substrate, were determined for the alkaline phosphatases of various tissues from different species of birds and mammals. These constants are compared with K_m values determined at a constant pH, the optimum for a concentration of substrate large enough to maintain zero-order rate of reaction.

METHODS AND MATERIALS

Methods for the preparation of plasma and tissue phosphatases and for the determination of phosphatase activity have been described previously (Motzok & Wynne, 1950; Motzok, 1950, 1959). With the exception of the study on pH optima without added Mg^{2+} ions (Fig. 3), phosphatase activities were determined in the presence of optimum amounts of $MgCl_2$ in the reaction mixtures, i.e. 5 mM for the phosphatases of plasma, bone, liver and kidney, and 0·01 M for the enzyme of intestinal mucosa. The strains and breeds used in this study were Sprague-Dawley ats, giant white rabbits, Yorkshire pigs, Chinese × Emrden goslings and Columbian Rock, Barred Plymouth Rock and New Hampshire fowls. The pigeons were of the common breeds. The goslings were raised on a practical type of diet (Branion & Hill, 1952). The other birds and the mamma were fed with commercial diets.

EXPERIMENTAL AND RESULTS

Influence of species of birds and mammals on pH *optima of intestinal phosphatase*

Fig. 1 shows that the phosphatase of intestinal mucosa of rats exhibited optimum activity with each concentration of β-glycerophosphate at the lowest pH values and the enzyme from the intestinal mucosa of swine had optimum activities at the highest pH values. There was little difference in the pH optima for the enzymes of goslings and of rats, on the one hand, and in the pH optima for the enzymes of pigeons and of swine, on the other. The pH optima for the enzyme of rabbits were somewhat lower than those for the enzyme of swine. In the avian class the intestinal enzyme of fowls (Columbian Rock) exhibited maximal activities at pH values which were intermediate between those required by the enzyme of goslings and those needed by the enzyme of pigeons.

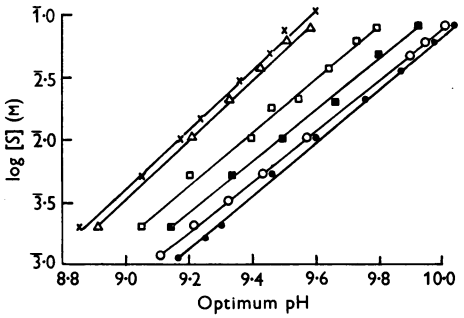

Fig. 1. Relationship between initial concentration of sodium β-glycerophosphate, expressed logarithmically, and pH for optimum alkaline phosphatase activity of intestinal mucosa of rats (×), goslings (△), fowls (□), rabbits (■), pigeons (○) and pigs (●). Activities were measured in the presence of 0·01 M-$MgCl_2$.

The data also show that a linear relationship exists between the logarithm of the initial concentration of substrate and the respective optimum pH for each enzyme preparation, the shift being to higher pH values with increasing concentrations of substrate. It was also found that the linear relationship between substrate concentrations, expressed logarithmically, and pH optima for the phosphatase of rat intestinal mucosa extended to physiological pH values with dilutions of substrate beyond the amounts shown in Fig. 1. With an initial concentration of 0·02 mM-substrate the optimum was pH 7·7. This confirms the findings of Ross *et al.* (1951) that with very dilute substrate the optimum pH for alkaline phosphatase is near neutral pH values.

pH *optima of phosphatases of different tissues*

Rat. Fig. 2 (*A*) shows that the pH optima for the enzymes of bone and kidney of rats were practically the same for each concentration of β-glycerophosphate, whereas the optima for the enzyme of liver were slightly lower. The differences in the pH optima for the phosphatases of plasma and intestinal mucosa were very small, if not negligible. These optima, however, were considerably below those for the enzymes of bone and kidney.

Rabbit. The phosphatases of liver and kidney of rabbits (Fig. 2*B*) were found to possess the highest pH optima with β-glycerophosphate recorded in these studies. In fact, the pH optima for high concentrations of substrate could not be determined because these appeared to be beyond the effective range of the buffer system employed. The pH optima for the phosphatases of bone and intestinal mucosa were considerably lower than those for the enzymes of liver and kidney, whereas plasma phosphatase exhibited maximum activities at intermediate values of pH.

Pigeon. The phosphatases of bone, kidney and intestinal mucosa of pigeons (Fig. 2*C*) appeared to possess comparable pH optima for the respective concentrations of β-glycerophosphate. On the other hand, the pH optima for the enzyme of liver were considerably lower than those required by the enzymes of the other tissues.

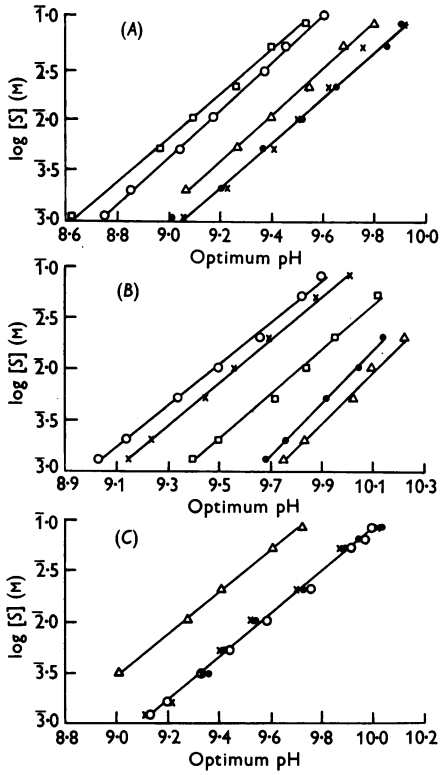

Fig. 2. Relationship between initial concentration of sodium β-glycerophosphate, expressed logarithmically, and pH for optimum alkaline phosphatase activity of plasma (□), intestinal mucosa (○), liver (△), kidney (●) and bone (×). (*A*) Rats; (*B*) rabbits; (*C*) pigeons. Activities were measured in the presence of optimum concentrations of $MgCl_2$ in the reaction mixtures: 5 mM for the phosphatases of plasma, bone liver and kidney and 0·01 M for the phosphatase of intestinal mucosa.

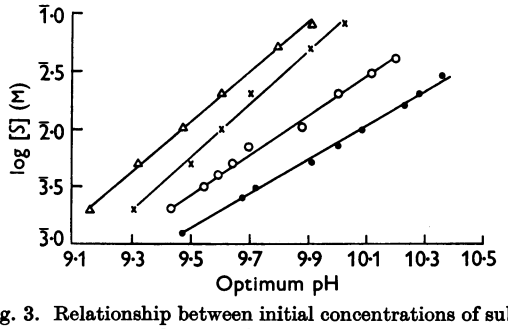

Fig. 3. Relationship between initial concentrations of substrate, expressed logarithmically, and the pH for optimum activities of alkaline phosphatase of intestinal mucosa of fowls (sodium β-glycerophosphate as substrate) in the presence of 0·01 M-$MgCl_2$ (△) and without added magnesium (×), and of plasma phosphatase of fowls (disodium phenyl phosphate as substrate) in the presence of 5 mM-$MgCl_2$ (●) and without added magnesium (○).

Influence of magnesium on pH *optima*

The data in Fig. 3 show the influence of added $MgCl_2$ on the pH optima required by alkaline phosphatase of plasma of fowls with phenyl phosphate as the substrate and by the enzyme of intestinal mucosa with sodium β-glycerophosphate. A linear relationship between the initial concentrations of substrate, expressed logarithmically, and the pH optima existed with and without added magnesium. However, the addition of magnesium to the reaction mixture changed the pH optima for both plasma and intestinal mucosa phosphatases, causing the optima to shift to higher values for the enzyme of plasma and to lower values for the enzyme of intestinal mucosa.

K_m values at optimum pH *for each concentration of substrate*

In studies on the kinetics of alkaline phosphatases of plasma and tissues of several species of birds and mammals, pH optima were determined for a wide range of concentration of substrate with each enzyme preparation. The activities of the phosphatases from various sources were measured at the optimum pH for each concentration of substrate and the velocities were plotted according to the method of Lineweaver & Burk (1934). The $1/v : 1/[S]$ relationship for all enzyme preparations was similar to those found previously (Motzok, 1959, figs. 4 and 8). The points which fell along straight lines were used for the calculation of the lines of best fit (Treloar, 1939). The constants, K_{m_1} for dilute substrate and K_{m_2} for substrate present in high concentrations, were derived from the calculations of the lines of best fit and are presented in Tables 1 and 2.

Twelve preparations of plasma phosphatase and six preparations of the enzyme of intestinal mucosa were obtained from several breeds of chicks used in diverse experiments and varying in age from 3 to 8 weeks. The average values for K_{m_1} and K_{m_2} (Table 1) were 2·1 mM and 22·5 mM for plasma phosphatase and 2·2 mM and 9·0 mM for the intestinal enzyme. Whereas the values for K_{m_1} for the phosphatases of the two sources were quite similar, the average values for K_{m_2} for plasma phosphatase were more than twice that for the enzyme of intestinal mucosa. Although K_{m_1} values for intestinal phosphatase of mature fowls were somewhat lower than those for the enzyme of chicks, the values for K_{m_2} were within the range of values found for the young birds. The data for chicks and mature birds were arranged in increasing order on the numerical values for K_{m_1} to show that variations in the respective values for K_{m_2} were not related to the variations in K_{m_1}.

Values for K_{m_1} for plasma phosphatases of goslings and chicks were about the same, whereas K_{m_2} for the plasma enzyme of goslings was only about one-tenth of K_{m_2} for the enzyme of chicks. Values for K_{m_1} and K_{m_2} for intestinal phosphatase of pigs and calves were comparable with those for fowls. The calf intestinal phosphatase was a commercial product, prepared by partial purification according to the method of Schmidt & Thannhauser (1943).

With disodium phenyl phosphate as the substrate for intestinal phosphatase of chicks, the values for K_{m_1} and K_{m_2} were lower than the corresponding constants obtained with β-glycerophosphate. With calf intestinal phosphatase, the value of 2 mM for K_{m_1} for phenyl phosphate was

Table 1. *K_m values of alkaline phosphatases from various sources*

Activities were determined at the respective pH optima for the different concentrations of substrate with each enzyme preparation. K_{m_1} and K_{m_2} values were calculated from activities with low and high concentrations of substrate respectively and plotted according to Lineweaver & Burk (1934).

	K_m (mM)					
	Sodium β-glycerophosphate				Disodium phenyl phosphate	
	Plasma		Intestinal mucosa		Intestinal mucosa	
Species	K_{m_1}	K_{m_2}	K_{m_1}	K_{m_2}	K_{m_1}	K_{m_2}
Chick	1·8	23	1·8	9	0·8	2·3
	1·9	15	1·9	9	0·9	2·3
	1·9	15	2·3	8	1·2	3·8
	1·9	17	2·3	12	1·3	3·2
	2·1	30	2·4	11		
	2·2	20	2·5	7		
	2·2	20				
	2·2	23				
	2·2	26				
	2·2	30				
	2·2	30				
	2·4	21				
Mature fowl	—	—	1·3	7	—	—
			1·5	6		
			1·5	6		
Goose	1·5	2·7	2·4	11	—	—
Pig	—	—	2·1	10	—	—
Calf*	—	—	1·7	11	2·2	†

* Calf intestinal mucosa purified commercially by the method of Schmidt & Thannhauser (1943).

† No estimate was possible because pH optima for high concentrations of phenyl phosphate were beyond the effective range of the buffer system.

Table 2. *K_m values of alkaline phosphatases of rat, rabbit, and pigeon*

Activities were determined at the respective pH optima for the different concentrations of sodium β-glycerophosphate with each enzyme preparation. K_{m_1} and K_{m_2} values were calculated from activities with low and high concentrations of substrate respectively and plotted according to Lineweaver & Burk (1934).

	K_m (mM)					
	Rat		Rabbit		Pigeon	
Source of enzyme	K_{m_1}	K_{m_2}	K_{m_1}	K_{m_2}	K_{m_1}	K_{m_2}
Plasma	1·3	16	*	*	*	*
Intestinal mucosa	1·6	9	1·2	5	2·4	11
Bone	1·6	11	1·9	11	1·9	10
Kidney	1·3	11	2·1	†	3·0	11
Liver	‡	‡	2·0	†	2·1	19

* No estimate was possible owing to low phosphatase activity.

† No estimate was possible because pH optima for high concentrations of substrate were beyond the effective range of the buffer system.

‡ No estimate was possible owing to high content of inorganic phosphate in the enzyme preparation.

comparable with the value obtained with β-glycerophosphate for the intestinal enzyme of other species. The pH optima for calf intestinal phosphatase were considerably higher with phenyl phosphate than with β-glycerophosphate (unpublished data) and activities with high concentrations of phenyl phosphate could not be determined because the pH optima appeared to be beyond the range of the buffer system employed.

In Table 2 are shown the values for K_{m_1} and K_{m_2} for the phosphatases of several sources in rats, rabbits and pigeons with β-glycerophosphate as the substrate. It appears that K_{m_1} values for intestinal mucosa and kidney phosphatases of pigeons were somewhat higher than those for the enzymes of the same sources in rats and rabbits, whereas values for K_{m_1} and K_{m_2} for bone phosphatase were quite similar in the three species.

The preparation of rat liver was found to contain an appreciable amount of inorganic phosphorus. This reduced the sensitivity of the method for determining the inorganic phosphate liberated enzymically and K_m values were not calculated. It was also observed that the phosphatase activity of the plasmas of pigeons and rabbits was very low and the method employed was inadequate to measure accurately differences in reaction velocities.

K_m values at a constant pH *for all concentrations of substrate*

K_m values for a number of preparations of phosphatase from several sources were also calculated from velocities measured at a constant pH for all concentrations of substrate. In each case the pH used was optimum for a concentration of substrate large enough to maintain zero-order rate of reaction. This study was made on the same preparations of enzymes used in obtaining the data in Tables 1 and 2 and the values presented in Table 3 can be compared directly with the data on the corresponding preparations recorded in Tables 1 and 2.

These studies show that, when the activities are measured at a constant pH for all concentrations of substrate, there is considerable variation in the calculated values for K_m for the phosphatases of various sources. In addition, these K_m values are in some cases four and five times the corresponding values for K_{m_1} calculated from velocities at the optimum pH for each concentration of substrate.

DISCUSSION

Factors affecting pH *optima*

The direct linear relationship between substrate concentration, expressed logarithmically, and optimum pH, reported by Ross *et al.* (1951) for the alkaline phosphatase of intestinal mucosa of rats, is exhibited by the alkaline phosphatases of various tissues of a number of species of birds and mammals. There are, however, marked differences in the pH optima for the enzyme of a specific tissue from different sources, e.g. intestinal phosphatase (Fig. 1), which cannot be related to differences in families, orders or even classes of vertebrates. In addition, there appears to be no explanation for some of the differences and similarities in pH optima for the phosphatases of various tissues in the same animal. For example, in pigeons (Fig. 2*C*) the optimum pH with each concentration of substrate was the same for the phosphatases of bone,

Table 3. *K_m values of alkaline phosphatases of various sources*

Activities were determined at one pH for all concentrations of substrate with each preparation of enzyme.

Substrate	Species	Source	pH*	K_m (mM)†
Sodium β-glycerophosphate	Mature fowl	Intestinal mucosa	9·7	7·6
		Intestinal mucosa	9·7	8·3
		Intestinal mucosa	9·7	10·9
	Pigeon	Intestinal mucosa	9·95	6·1
		Bone	9·95	11·0
		Kidney	10·0	14·8
		Liver	9·8	5·1
	Rabbit	Intestinal mucosa	9·9	2·6
		Bone	9·98	10·7
		Kidney	10·1	6·7
		Liver	10·16	5·4
	Calf	Intestinal mucosa	9·9	11·5
	Pig	Intestinal mucosa	9·95	8·8
Disodium phenyl phosphate	Chick	Intestinal mucosa	10·0	3·1
	Calf	Intestinal mucosa‡	10·0	4·0

* Optimum pH for a concentration of substrate large enough for zero-order reaction rate.
† Calculated from activities plotted according to Lineweaver & Burk (1934).
‡ Calf intestinal mucosa purified commercially by the method of Schmidt & Thannhauser (1943).

kidney and intestinal mucosa, whereas in rats (Fig. 2*A*) the pH optima were the same for the phosphatases of bone and kidney and markedly different for the enzyme of intestinal mucosa. In the chick it was found (Motzok, 1950) that the pH optima were the same for the phosphatases of bone, kidney, liver and plasma, whereas the optimum pH for the enzyme of intestinal mucosa was considerably lower. In the present study the data show that in rats the pH optima for the enzymes of plasma and intestinal mucosa are quite similar (Fig. 2*A*).

Bodansky (1934) concluded that the source of phosphatase in blood was of diverse origin. In diseases of liver and bone he considered the increase in serum phosphatase to arise from the particular organ that was involved. Kay (1933) had suggested that the phosphatase of serum was identical with that of various tissues, being the result of leakage into the circulatory system from tissues of higher phosphatase content. Motzok (1950) presented evidence that in rachitic chicks the skeleton is the main source of the increased plasma phosphatase. The similarity in the pH optima for the phosphatases of plasma and intestinal mucosa in rats (Fig. 2*A*) lends support to the suggestion of Flock & Bollman (1948) and Madsen & Tuba (1951) that in the rat the alkaline phosphatase is supplied to the plasma by the small intestine.

If the phosphatase of plasma in rats originates in the intestinal mucosa, the similarity in pH optima for the enzymes of the two sources suggests that the environmental medium of the plasma did not contain factors which altered the characteristics of the enzyme governing the pH required for optimum activity *in vitro* under the conditions of the experiment. The data in Fig. 3, however, show that the addition of magnesium may exert an opposite effect on pH optima for phosphatase of different sources, e.g. raising the pH optima for the phosphatase of plasma and lowering the pH optima for the enzyme of intestinal mucosa of fowls. It was shown previously (Motzok, 1945) that the addition of magnesium also caused the pH optima for plasma phosphatase of chicks to shift to higher values with β-glycerophosphate. Therefore the shift in pH optima in opposite directions for intestinal phosphatase with β-glycerophosphate and plasma phosphatase with phenyl phosphate cannot be attributed to the type of substrate used in measuring phosphatase activity. The addition of magnesium does not change the pH optima of alkaline phosphatase of all sources, as was shown by Aebi & Abelin (1948), who found that added magnesium had no effect whereas added manganese caused a definite shift in optimum pH for the unpurified enzyme of rat kidney. It appears that the factors which determine the optimum pH of a particular phosphatase reside in the nature of the enzyme. Consequently, the manner and degree of the influence of environmental factors are probably governed by the characteristics of the enzyme itself. There remains for further study the effect of activators on the pH optima for phosphatases of other sources.

The present findings serve to emphasize the fallacy of applying optimum conditions established for the phosphatase of one source to the phosphatases of other sources. Consideration of all known factors is desirable in order to avoid further confusion in the literature with respect to pH optima of alkaline phosphatase, as indeed of other enzymes.

Factors influencing K_m values

The problem of the choice of pH for measuring phosphatase activity with a range of concentration of substrate large enough for an accurate estimation of K_m has been discussed (Motzok, 1959). The data in Table 3 show that there was considerable variation in K_m derived from activities measured at a constant pH for all concentrations of substrate. At least a part of the variation in K_m derived in this manner was found to be caused by variation in the amount of enzyme in the reaction mixture (Motzok, 1959). Even if the amount of enzyme employed were kept constant on the basis of activity, one cannot safely assume that the pH–activity curves with each concentration of substrate are the same for the phosphatases from all sources. Certainly, the use of one pH for all phosphatases would yield K_m values which would be affected to an appreciable extent by differences in pH optima which exist among species and tissues of individual species. The influence of other factors, such as buffers and activators, on pH optima would also have to be taken into consideration.

Van Slyke (1942) has pointed out that, although K_m is a useful value, it can be called a constant only for defined conditions; e.g. a drop of 1 unit in pH will increase the K_m of urease 11-fold (Van Slyke & Cullen, 1914). Similarly, K_m values for phosphatases, which have been recorded in the literature, can at best be regarded as being applicable only to the particular preparation of enzyme used and for the specific set of conditions *in vitro* under which the activities were measured. Unfortunately, in many instances no details of the methods employed have been recorded. In cases where such information has been given, the direct comparison of K_m values, as distinguishing characteristics of phosphatases, could be made only with due regard to various factors such as those mentioned above.

It was shown previously (Motzok, 1959) that the effect of the amount of enzyme in the reaction

mixture on K_m, when the velocities were measured at a constant pH, was largely eliminated by measuring velocities at the optimum pH for each concentration of substrate. Indeed, there was only a small variation in the values for K_{m_1} and K_{m_2} for the phosphatases of a number of preparations of plasma and intestinal mucosa of chicks (Table 1) when the optimum pH for each concentration of substrate was used. Considerably more data are required on the phosphatases of other sources with different substrates to evaluate fully the differences and similarities in the values for K_{m_1} and K_{m_2} reported in this paper.

SUMMARY

1. Marked differences, and in some cases unexpected similarities, were found in the pH optima (for the respective concentrations of substrate) required for the activities of the alkaline phosphatases from various tissues of different species of birds and mammals.

2. Linear relationships were shown to exist between the initial concentrations of substrate, expressed logarithmically, and the pH optima for the activities of the phosphatases from various sources. The optimum pH shifted to higher values with increasing concentration of substrate.

3. The addition of magnesium concentrations (5 mM for plasma phosphatase and 0·01 M for intestinal phosphatase) for maximum activation caused the respective pH optima to shift to higher values for the plasma phosphatase and to lower values for the intestinal enzyme of fowls when compared with pH optima without the addition of magnesium.

4. By the use of velocities at the optimum pH for each concentration of substrate, values for K_{m_1} from data with dilute substrate, and K_{m_2} from data with high concentrations of substrate, were calculated for the phosphatases of various tissues and plasma of fowls, geese, pigeons, rats, rabbits, pigs and calves.

5. The average values for K_{m_1} and K_{m_2} with β-glycerophosphate were 2·1 mM and 22·5 mM for 12 preparations of plasma, and 2·2 mM and 9 mM for six preparations of intestinal mucosa of chicks. With phenyl phosphate, the average values for K_{m_1} and K_{m_2} for four preparations of intestinal mucosa of chicks were 1 and 3 mM. Variations in the values for K_{m_1} and K_{m_2} were quite small. More data are required on the phosphatases of other sources with different substrates to evaluate the similarities and differences in the constants recorded.

6. There was considerable variation in K_m derived from velocities measured at a constant pH for all concentrations of substrate.

The authors wish to thank Miss G. Ritcey and Mr G. Amos for valuable technical assistance.

REFERENCES

Aebi, H. & Abelin, I. (1948). *Helv. chim. acta*, **31**, 1943.
Bodansky, A. (1934). *J. biol. Chem.* **104**, 473.
Branion, H. D. & Hill, D. C. (1952). *Poult. Sci.* **31**, 1100.
Delory, G. E. & King, E. J. (1943). *Biochem. J.* **37**, 547.
Flock, E. V. & Bollman, J. L. (1948). *J. biol. Chem.* **175**, 439.
Hofstee, B. H. J. (1955). *Arch. Biochem. Biophys.* **59**, 398.
Kay, H. D. (1933). *Physiol. Rev.* **12**, 384.
Lineweaver, H. & Burk, D. (1934). *J. Amer. chem. Soc.* **56** 658.
Madsen, N. B. & Tuba, J. (1951). *J. biol. Chem.* **195**, 741.
Morton, R. K. (1955). *Biochem. J.* **60**, 573.
Morton, R. K. (1957). *Biochem. J.* **65**, 674.
Motzok, I. (1945). Ph.D. Thesis: University of Toronto.
Motzok, I. (1950). *Biochem. J.* **47**, 193.
Motzok, I. (1959). *Biochem. J.* **72**, 169.
Motzok, I. & Wynne, A. M. (1950). *Biochem. J.* **47**, 187.
Ross, M. H., Ely, J. O. & Archer, J. G. (1951). *J. biol. Chem.* **192**, 561.
Sadasivan, V. (1952). *Nature, Lond.*, **169**, 418.
Schmidt, G. & Thannhauser, S. J. (1943). *J. biol. Chem.* **149**, 369.
Treloar, A. E. (1939). *Elements of Statistical Reasoning* p. 108. New York: J. Wiley and Sons Inc.
Van Slyke, D. D. (1942). *Advanc. Enzymol.* **2**, 33.
Van Slyke, D. D. & Cullen, G. E. (1914). *J. biol. Chem.* **19**, 141, 211.
Walker, P. G. & King, E. J. (1950). *Biochem. J.* **47**, 93.
Zittle, C. A. & Della Monica, E. S. (1950). *Arch. Biochem.* **26**, 112.