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Studies on organ-specific alkaline phosphatases in relation to its diagnostic value

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With 4 figures

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Several investigations have established that individual plasma and tissue enzyme activities are composed of two or more similar but chemically, immunologically and electrophoretically different components. *Keiding* (1), *Hadson, Latner & Raine* (2) using electrophoretic techniques achieved fractionation of serum alkaline phosphatases. It has also been reported by *Markert* (3) that tissues contain one or more of these isoenzymes.

Using tissue isoenzyme differences and the fact that the different pathological states cause changes in the isoenzyme patterns; it has been possible to correlate the disease state of various organs with plasma isoenzyme pattern changes.

Boyer, Schultz & Weilbacher (4) reported that tissue sources of various enzymes can possibly be established by comparing them with enzymes from different tissues. In the same line, *Bruns* (5) suggested that in order to evaluate and differentiate an organ specific enzyme, several points must be taken into consideration. These are the role of pH, enzyme kinetics, electrophoretic behaviour, selective activators or inhibitors as well as immunochemical procedures.

The significant variations in serum alkaline phosphatases activities in biliary and bone diseases are already well established by *Armstrong, King & Harris* (6) and *Bodansky & Jaffe* (7).

The aim of the present work is thus to study the role of pH on tissue alkaline phosphatases of different organs of human subgests. The organs selected are liver, bone, intestine, kidney and adrenals, since these organs represent the richest sources of alkaline phosphatases as previously reported (8). In this respect, tissue alkaline phosphatase activities were determined at different pH values ranging from pH 8.0 to pH 10.6.

Material and methods

Tissue extracts

For this purpose, normal human adult tissues without any apparent diseases, were obtained from autopsy within 24 hours after accidental death were included. After being freed from fat and other extraneous tissues, they were washed with physiological saline, then with cold water till being free from blood cells. They were plotted by filter paper and homogenised in a glass homogeniser with physiological saline in the proportion of 1:20 with the addition of 10 % toluene. Tissues were left to autolyse at room temperature for two days and then kept in the cold for further 24

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hours and finally centrifugued for 15 minutes at 3.000 r.p.m. The supernatants thus prepared keep their activities unchanged for several monthes if kept in a refrigerator.

Determination of pH hydrolysis curves:

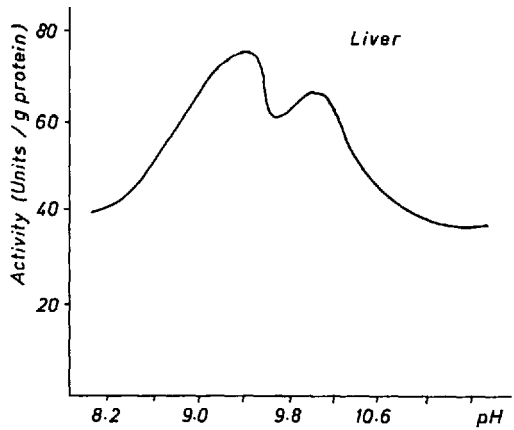
The method used for determination of alkaline phosphatase activities was that described by *King, Abul-Fald & Walker* (9). Using disodium phenyl phosphate as a substrate and veronal-carbonate buffer covering a pH range from 8.0 to 10.6 pH of each solution was checked by pH meter. After mixing equel volumes of 0.02 M substrate, disodium phenyl phosphate and veronal-carbonate buffer (2.0 ml each), the pH was again adjusted, warming the mixture to 37 °C in a water bath; 0.2 ml of tissue homogenate were added and allowed to hydrolyse for exactly 30 minutes. At the end of the encubation period, 1.8 ml of Folin-Phenol reagent were added to stop the reaction and precipitate proteins. Phenol liberated was estimated colourimetrically.

Enzyme units were expressed as mg phenol liberated/30 minutes/1 g tissue protein at 37 °C.

The protein content of the homogenates were determined by the micromethod described by *Lowry* (10).

Results and discussion

The pH hydrolysis curves of different tissue alkaline phosphatases were presented in the accompanying graphs.



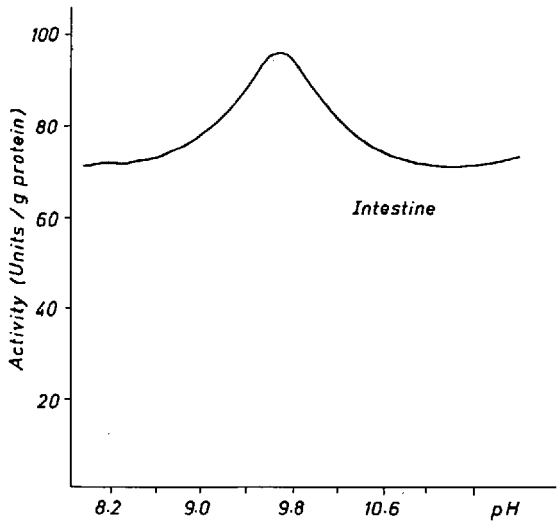
The pH hydrolysis curves showed that liver tissues contain two alkaline phosphatase isoenzymes; one optimally active at pH 9.2–9.4 and the other at pH 9.9. The first isoenzyme activity is higher than the second and its optimal pH was shown to vary slightly with different individuals.

Bone was found to contain two isoenzymes optimally active at pH 9.4 and 9.9 respectively and the activity at pH 9.9 was comparatively lower than that at pH 9.4 similar to that observed in case of liver tissues.

From the above mentioned observations, it is clear that liver and bone alkaline phosphatases are very similar in the shape of pH hydrolysis curves. This similarity agreed well with the study carried out by *Moss, Diana, Karkas & King* (11) on the enzyme kinetics where they failed to reveal any differences in serum alkaline phosphatases in cases suffering

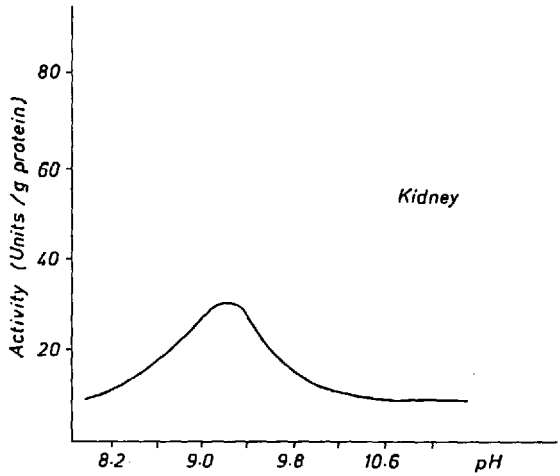
from bone and biliary diseases. *Rosenberg* (12), using zone electrophoresis, two zones for serum alkaline phosphatases were obtained in some cases suffering from disorders associated with increased serum alkaline phosphatases activity. The two zone obtained by *Rosenberg* had a single optimal pH at 9.8 which is very near to our second optimal pH, 9.9 obtained in the present work. Also, the two zones were not specific for neither bone nor biliary diseases. The similarity of liver and bone isoenzymes extends to the proportion of the two isoenzymes obtained. These ratios were (1.14 : 1.00) and (1.15 : 1.00) at pH 9.4 and 9.9 respectively for both bone and liver tissues.

The presence of two enzymes in liver tissues supported the claim of *Cloetens* (13) that liver contains two alkaline phosphatases.

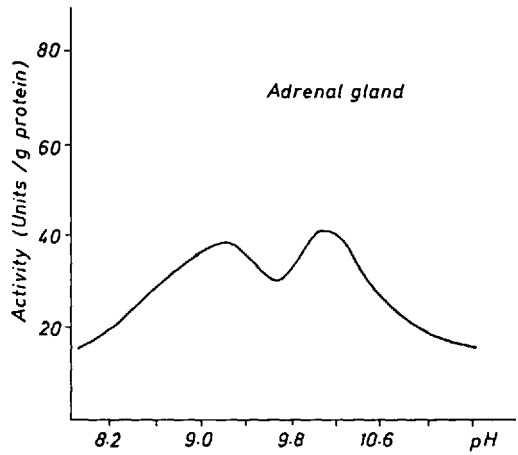


The kidney contains only one alkaline phosphatase optimally active at pH 9.0–9.2 which showed a slight variation with different individuals. In this connection, *Bonting, Pollak & Meuhrcke* (14) using histochemical techniques reported that kidney alkaline phosphatase was optimally active at pH 10.0. These variations may be due to differences in techniques, buffers and substrates used; since these factors are known to influence the optimal pH of enzyme activities.

The adrenal gland, in addition to and isoenzyme of optimal pH 9.2 it contains another isoenzyme optimally active at pH 10.1 which was absent from kidney tissues. This finding is very interesting since *Bonting* reported that kidney alkaline phosphatase was optimally active at pH 10.0. Thus the origin of such enzyme may be the adrenal gland and not the kidney especially those workers did not consider the adrenal gland during their studies. It is worthy to note that the adrenal gland is by far more rich in alkaline phosphatase activity when compared with the kidney tissues and the isoenzyme optimally active at pH 10.1 corresponds to about three folds the activity at pH 9.2 in the adrenal gland.



Intestinal mucosa, although it is rich in alkaline phosphatase yet it contains only one enzyme which is optimally active at 9.7. Intestinal alkaline phosphatase seems to be unique in its optimal pH in being specific and sharp. This property of intestinal alkaline phosphatase agreed with the findings of *Fishman* (15) who reported the stereospecific inhibition of intestinal alkaline phosphatase by L-phenylalanine rather than other tissue alkaline phosphatases.



Summary

The role of pH on tissue alkaline phosphatases have been investigated in different human tissues. Different isoenzymes have been observed in different organs. Bone and liver were similar in having the isoenzymes optimally active at 9.4 and pH 9.9. The kidney has a single alkaline phosphatase optimally active at pH 9.2, while the adrenal gland has two isoenzymes of optimum pH 9.2 and 10.1. Intestinal alkaline phosphatase was optimally active at pH 9.7.

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