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THE EFFECT OF ADMINISTRATION OF THYROID HORMONE ON THE ALKALINE PHOSPHATASE IN THE MOUSE'S SERUM

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Although many investigators have shown that the activity of the alkaline phosphatase in serum is increased by thyroxine administration (KAY, 1930; PELCZAR and ST. MURZA-MURZICZ 1937; TALBOT, 1939), it must be noticed that not every administered individual showed such an increase, and that it was not always found in all substrates concerned with this enzymatic reaction (PELCZAR and ST. MURZA-MURZICZ, 1939). On the other hand, the presence of at least two distinguishable alkaline phosphatases was demonstrated in the rat's kidney and in several other tissues (CLOETENS, 1939; GRyder, FRIEDENWALD and CARLSON, 1955). In addition, it must be considered that the metabolic change due to the administration of thyroid hormone may be a cause of the so called kinetic response of the enzymatic reaction (KNOX, AUERBACH and LIN, 1956).

It is, therefore, necessary to show what kinetic changes of the enzymatic reaction take place in the serum of a hormone-administered animal, as well to study the change of enzymatic activity which has been thought to consist merely of a changed concentration of enzyme.

In the present work, the effect of substrate concentration and magnesium ion concentration as an activator on the phosphatase activity in mouse's serum for p-nitrophenyl phosphate in an alkaline environment, was studied in an attempt to find what alteration in enzymatic reaction was produced by the administration of thyroid hormone.

Experimental

Animals: DD strain male mice weighing 15-18 gm. were used. The administration of thyroid hormone was as follows; thyroid tissue extract (0.1 ml. of Thyradine by Teikoku Zoki Co. Ltd.) was injected subcutaneously in the back

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of a mouse (THI) or 40 mg. desiccated thyroid powder (Thyradine) suspended in a small amount of milk was orally administered (THF). Extract (0.1 ml.) was equivalent to 20 mg. desiccated thyroid. The animals thus treated were sacrificed 6 hours after the administration.

The sample sera were prepared by centrifuging (2000 r.p.m.) the blood obtained through an incision in the carotid. Samples were stored in ice water at once. No attempt was made for further purification.

Assay of enzymatic activity: The method of BESSEY, LOWRY and BROCK (1946) was adopted in principle, however, modification was made to keep the concentration of serum in the incubation mixtures as low as possible, since the influences of some factors other than the enzyme in serum could be neglected at the hydrolyzing reaction. Standard procedure was as follows: For the reaction system, 1 ml. of $MgCl_2$ (2.5×10^{-3} – 1.0×10^{-4} M) and 2 ml. of Veronal-HCl or Veronal-Bicarbonate buffer (0.05 M) (KING and DERLOY; 1940) and 1 ml. of disodium p-nitrophenyl phosphate solution were mixed in the 80 mm. \times 7 mm. serological test tube. For incubation a Warburg constant temperature water bath, at 37°C., equipped with a test tube stand was employed.

After ten minutes' temperature equilibrium of the system, 0.02 ml. of serum was added to the system by LANG-LEVY'S constriction pipette and mixed quickly and thoroughly. At measured intervals of time aliquotes of the mixture were pipetted into 2 ml. of 1% NaOH to stop the reaction. Then the intensity of yellow colour of the liberated p-nitrophenolate was determined colorimetrically by means of Hitachi's spectrophotometer at the wavelength of 405 μ . As the

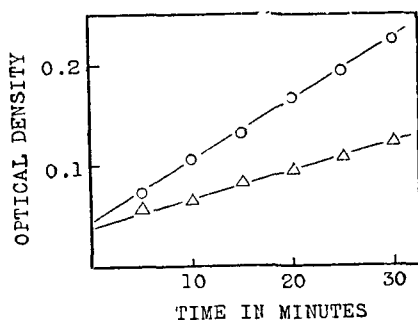


Fig. 1. Time curves of mouse-serum alkaline phosphatase for p-nitrophenyl phosphate.

Incubation mixture: 2.5×10^{-3} M Mg^{++} , 2.5×10^{-3} M Substrate (disodium p-nitrophenyl phosphate), 0.025 M Veronal-HCl Buffer at pH 9.7.

—○— 0.04 ml. serum/4 ml. mixture.

—△— 0.02 ml. serum/4 ml. mixture.

increase of optical density was linear for first 30 minutes, the reaction velocity was obtained from the slope of the increase of the optical density within 20 minutes (Fig. 1).

BESSEY, LOWRY and BROCK's unit could be calculated from these values but it was not calculated since no attempt was made to study the change of enzymatic activity itself and to compare it to other results. Each figure was based on the tests of 5 or more individuals in which the same results were obtained.

Results and Discussion

Effect of pH: The pH-activity curves are shown in Fig. 2. The optimum pH determined under the conditions illustrated in Fig. 2, is 10.0 in normal and THI or THF sera. No difference could be seen in the pH-activity for both cases in shape, though the latter one showed a narrower curve.

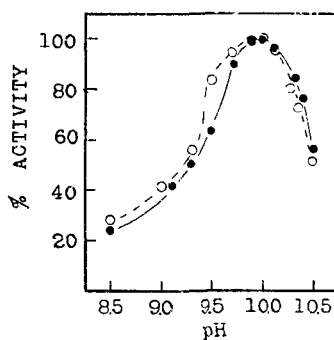


Fig. 2. pH activity curves of normal (open circle) and THF (block circle) sera.
Incubation mixture: 2.5×10^{-3} M Mg^{++} , 2.5×10^{-3} M Substrate, 0.025 M Vernal-bicarbonate buffer, 0.02 ml. serum/4ml. mixture.

By SHINOWARA and JONES (1942) it was summarized that the species difference in optimum pH of serum alkaline phosphatase is present and for different substrates, MORTON (1955) has reported that the optimum pH of enzyme prepared from milk was 10.05 for phenylphosphate, while it was 9.65 for glycerophosphate.

By GRYDER, FRIEDENWALD and CARLSON (1955) optimum pH of B enzyme in rat's kidney (activated by Mg^{++} , inhibited by Zn^{++}) was lower than A enzyme (activated by glycine and Zn^{++}) in the absence of added Mg^{++} . However, upon addition of Mg^{++} the optimum pH of B enzyme shifted toward alkaline side.

Then the effect of Mg^{++} on the optimum pH was studied in the normal serum (Fig. 3 A) and THF serum (Fig. 3 B). For the removal of Mg^{++} from the system, $MgCl_2$ sol. was replaced with EDTA sol. as illustrated in the Fig. 3. Mg^{++} activation was observed in both sera, though it was stronger in the normal serum. In normal as well as THF serum, upon the addition of Mg^{++} the optimum pH was shifted toward the alkaline side about 0.3 of a pH unit similarly to GRZYDER'S B enzyme.

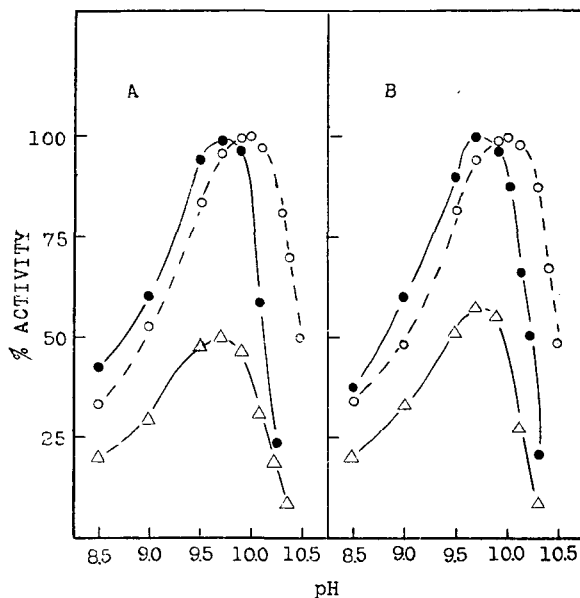


Fig. 3. pH curves of normal serum (A) and THF serum (B) in relation to Mg^{++} activation.

Incubation mixture: 2.5×10^{-3} M Substrate, 0.025 M Veronal-bicarbonate buffer, and - - ○ - - 2.5×10^{-3} M Mg , -●- 5×10^{-3} M EDTA. -△- % activity to the highest of the Mg^{++} addition system.

It may be considered here that in both sera a single enzyme is dominant at least referring to the pH-activity curve.

Effect of substrate concentration: The influence of varying substrate concentration was tested in the presence of a fixed concentration of Mg^{++} (2.5×10^{-3} M) at pH 9.7.

Reciprocal of the reaction velocity was a linear function of the reciprocal of substrate concentration, yielding an apparent Michaelis constant K_s .*)

*) Under the Michaelis-Menten-Briggs-Haldane mechanism $E + S \xrightleftharpoons[k_{-1}]{k_1} ES \xrightarrow{k_2} E + P$
 $1/v = (K_M/V_m) \cdot (1/[S]) + 1/V_m.$

The apparent Michaelis constant was the same, 1.33×10^{-4} M, for every normal individual. However, with the injection of the thyroid extract the individual variation in the apparent Michaelis constant centering around the value of normal individual come out and last as long as for 48 hours (Fig. 4). From this fact it is conceivable that the mechanism of hydrolysis was changed by an administration of thyroid hormone if the accordance at the case of normal serum was not a mere coincidence (10 individuals).

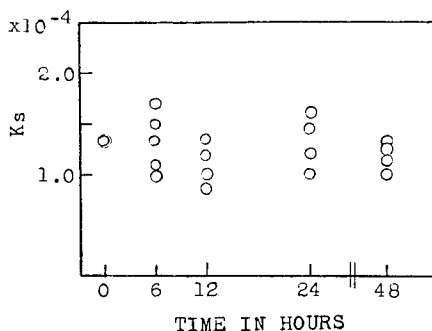


Fig. 4. Effect of thyroid hormone-injection on the apparent MICHAELIS constant of enzymatic activity obtained from Lineweaver-Burk plots.

For the normal serum, the apparent Michaelis constant was unaffected by dilution of enzyme (serum) or buffer in reaction mixture (Fig. 5).

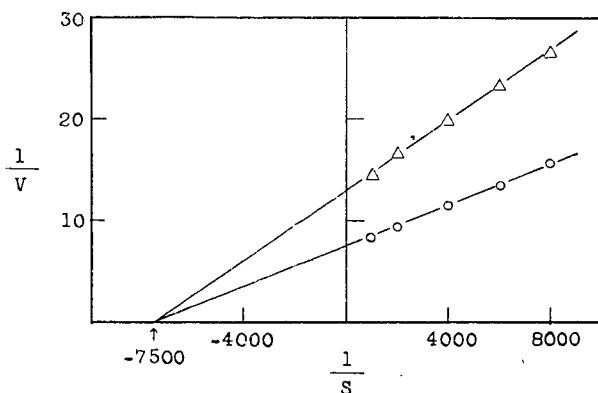


Fig. 5. Normal serum enzyme: Varying concentration of substrate and different levels of serum. Veronal-HCl buffer at pH 9.7.
 —○— 0.04 ml. of serum/4 ml. mixture.
 —△— 0.02 ml. of serum/4 ml. mixture.

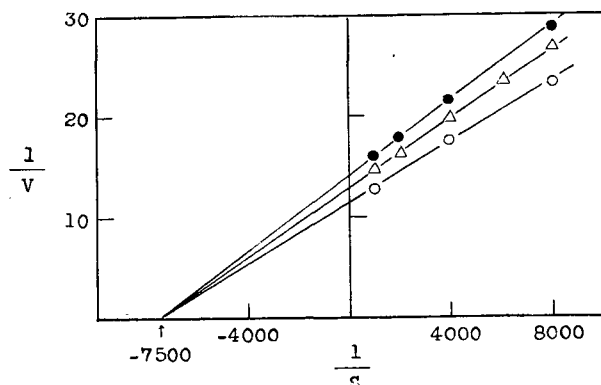


Fig. 6. Normal serum enzyme: Varying concentration of substrate and different levels of Mg^{++} , 0.025 M Veronal-HCl buffer at pH 9.7.

- $2.5 \times 10^{-3} \text{ M Mg}^{++}$.
- △— $5.0 \times 10^{-4} \text{ M Mg}^{++}$.
- $1.0 \times 10^{-5} \text{ M Mg}^{++}$.

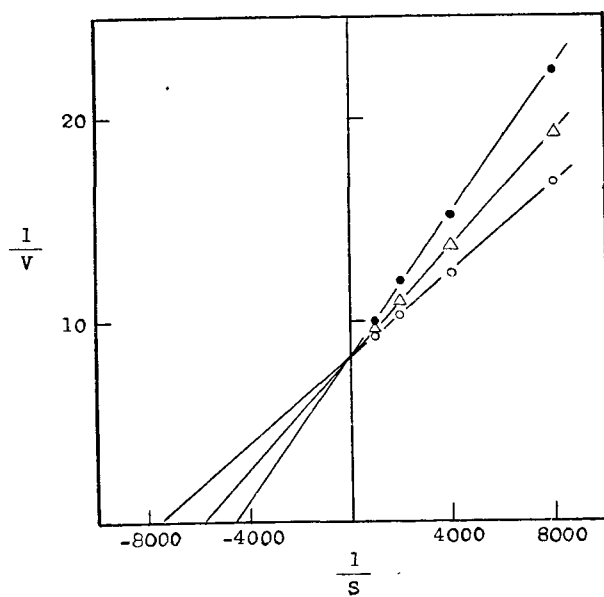


Fig. 7. THF serum enzyme: Varying concentration of substrate and different levels of Mg^{++} , 0.025 M Veronal-HCl buffer at pH 9.7.

- $2.5 \times 10^{-3} \text{ M Mg}^{++}$.
- △— $5.0 \times 10^{-4} \text{ M Mg}^{++}$.
- $1.0 \times 10^{-5} \text{ M Mg}^{++}$.

The influence of Magnesium ion: By the addition of MgCl_2 sol. at various concentration (from 10^{-5} M to 10^{-3} M), the initial pH of disodium p-nitrophenyl phosphate sol. (10^{-3} M) was not affected. Then it may be concluded that the substrate and MgCl_2 were non-interactive.

In normal serum, the apparent Michaelis constant was also found to be unaffected by variation in the concentration of Mg^{++} (Fig. 6). On the other hand, in THI or THF serum V_{\max} was not affected by variation in concentration of Mg^{++} , while the apparent Michaelis constant was changed (Fig. 7).

From these results it may be accepted that the apparent Michaelis constant is the true Michaelis constant for normal serum; however by an administration of thyroid hormone the mechanism in which the reaction was activated by Mg^{++} is changed, then the apparent Michaelis constant for the THI or THF serum is not accepted as the true Michaelis constant. For the normal serum, under the Michaelis-Menten-Briggs-Haldane mechanism,

$$\text{in } \quad Ks = (k_{-1} + k_2)/k_1 \\ k_{-1} \gg k_2 \quad \text{then} \quad Ks = k_{-1}/k_1$$

On the other hand, for THF serum

$$k_{-1} \ll k_2 \quad \text{then} \quad Ks = k_2/k_1$$

If it is supposed that the enzyme has two sorts of active sites from one of which the product is liberated and in another the substrate-enzyme complex is formed, the following explanation is possible. In normal serum the magnesium ion is required for activation of the liberating sites of the enzyme; then the V_{\max} is affected by variation in Mg^{++} concentration. However in the THF serum, the magnesium ion is required for the activation of the substrate-binding site of the enzyme; then the apparent Michaelis constant is varied by variation of Mg^{++} concentration.

Full kinetical analysis and physiological meaning of this enzymatic reaction will be reserved for a future study.

Summary

The effect of an administration of thyroid hormone on the mouse's serum-alkaline phosphatase for p-nitrophenyl-phosphate was studied.

In both normal and administered sera the optimum pH was 9.7 in the absence of magnesium ion, however, it was shifted toward the alkaline side by the addition of magnesium ion.

In normal serum the apparent Michaelis constant was the same for different individuals, and it was unaffected by variation in the concentration of all the components of the reaction system.

In the administered mouse's serum the apparent Michaelis constant has individual difference and it was affected by variation of the magnesium ion concentration in the reaction system.

Magnesium ion was found to be an activator for normal serum over the whole range of substrate concentration. However for the administered mouse's serum, the activation by magnesium ion was decreased with the increase of substrate concentration, and finally at the V_{max} , the effect of variation in magnesium ion concentration could not be observed.

Then it was revealed that only for normal serum may the apparent Michaelis constant be acceptable as the true Michaelis constant. Also, the mechanism of magnesium activation in vitro was altered in the mouse's serum by an administration of thyroid hormone.

Acknowledgement

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