THE ESTIMATION OF HIGH CONCENTRATIONS OF PLASMA ACID PHOSPHATASE

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Plasma acid phosphatase estimation is of established value in the diagnosis of carcinoma of the prostate with bone secondaries, and in the assessment of stilboestrol treatment (Gutman and Gutman, 1938; Herbert, 1946; Wray, 1956).

Modifications of the method of Gutman and Gutman (1938), using disodium phenyl phosphate substrate, and estimating the phenol liberated with Folin and Ciocalteu reagent, are widely used. When the result exceeds 40 units/100 ml. it is suggested that the estimation be repeated with diluted plasma (Varley, 1954). Herbert (1946) reduced the time of incubation as an alternative.

Because of the low Michaelis constant for acid phosphatase, approximately 0.0006 (Schönheyder, 1952) and the relatively high concentration of substrate used in the standard technique, a linear relationship between enzyme concentration and activity would be expected over a wider range than the accepted range of 0 to 40 units/100 ml.

The final blue complex produced by phosphotungstic/phosphomolybdic acids, phenol, and alkali, "goes to completion only in the presence of a gross excess of reagent and alkali" (Folin and Ciocalteu, 1927), so that dilution of the final coloured solution with water alone is obviously unsatisfactory. In the present investigation, dilution with reagent instead of with water extended this range of linearity.

Experiments

Reagents.—These are heparinized plasma stored at 2 to 4° C., and a prostatic extract made by grinding a normal prostate in sand with saline. Coarse particles were allowed to settle, and the supernatant fluid diluted in saline.

Methods.—The acid phosphatase activity of serial dilutions of plasma and prostatic extracts was estimated by the method of Gutman and Gutman (1938) as modified by King (1946), employing a final solution volume of 10 ml. to facilitate reading in an EEL

photoelectric absorptiometer. When the reading of the test solution exceeded 80, it was diluted 1/5, 1/10, or 1/20 according to the colour intensity. The diluents used were distilled water and, added successively, Folin and Ciocalteu reagent and sodium carbonate and water to maintain the concentration of these reagents. The addition of sodium carbonate was found necessary, for the final concentration must be kept above 3% for maximum colour development. The actual details are as follows:

Addition	Dilution		
	1/5	1/10	1/20
Final coloured solution (ml.) . .	2.0	1.0	0.5
Folin and Ciocalteu reagent 1/3 (ml.) . .	1.0	1.1	1.15
Sodium carbonate solution 15% (ml.) . .	1.6	1.8	1.9
Distilled water (ml.)	5.4	6.1	6.45

After 15 minutes at 37° C. the colour was measured against the blank and standard which had been left at room temperature. Their colour intensity does not change in one hour.

Results

Dilutions of plasma with an acid phosphatase of 720 units/100 ml. from a case of prostatic carcinoma gave similar results to dilutions of prostatic extract. Fig. 1 shows that dilution of the final colour with water was unsatisfactory, for there was no linear relationship between dilution and result (Curve A). By adding Folin and Ciocalteu reagent, together with alkali, linearity was obtained up to approximately 300 units/100 ml.

Discussion

According to the Michaelis and Menten theory, at high substrate concentration the rate of hydrolysis of substrate is proportional to the concentration of enzyme. As substrate is utilized the process finally becomes a first order reaction in which

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Schönheyder (1952), using a continuous titration method, has shown that the kinetics of acid phosphatase do not appear to follow the simple Michaelis and Menten theory, and that phosphate is inhibitory.

lysis during collection may be neutralized by formaldehyde (Abul-Fadl and King, 1948); (c) phosphatase of non-prostatic origin may contribute to the result as in Paget's disease (Sullivan, Gutman, and Gutman, 1942), and may be distinguished, by its alcohol resistance, from prostatic phosphatase (Herbert, 1946); (d) con-

FIG. 1.—Activity/enzyme concentration curves. B=●—●=Final coloured solution diluted with reagent and alkali. A=X—X=Final coloured solution diluted with distilled water.

Using the standard technique of King (1946), and within the limits of routine experimental error, a linear relationship exists between activity and enzyme concentration up to enzyme concentrations of approximately 300 units/100 ml. The limiting factor in this relationship would appear to be colour development by the Folin and Ciocalteu reagent and not inhibition by the products of reaction or exhaustion of substrate, according to the simple Michaelis and Menten theory.

Many causes of misleading high results have been recorded, e.g. (a) prostatic massage (Hock and Tessier, 1949) and retention of urine (Wray, 1956), possibly from trauma during catheterization; (b) red cell phosphatase liberated by haemo-

tamination of the citrate buffer with phosphatase-producing bacteria may also be a cause of error (Herbert, 1946).

The recording of results which might be too low has led to less comment. Failure to recognize the upper limit of colour development reduces the apparent maximum result to the order of 200 units/100 ml. Rapid decay of enzyme activity at 37° C. in neutral solution will introduce a variable error if the plasma is warmed in the water-bath before it is added to the buffer/substrate, in which it is stable.

Summary

Plasma acid phosphatase estimation by the method of Gutman and Gutman (1938, see

King, 1946), using phenyl phosphate substrate and Folin and Ciocalteu reagent, is reliable with enzyme concentrations up to 30 units/100 ml. This limit may be raised to 300 units/100 ml. by dilution of the final coloured solution with Folin and Ciocalteu reagent and sodium carbonate solution. Above this concentration the estimation must be repeated, either with diluted plasma or using a shorter incubation time.

Other causes of error are reviewed.

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