CLIII. THE EFFECT OF ASCORBIC ACID ON β -GLYCEROPHOSPHATE

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In a previous paper [1937] we reported that ascorbic acid activated serum phosphatase, as shown by the increase in inorganic phosphate in the presence of sodium β -glycerophosphate. The accompanying paper by King & Delory [1938] reports experiments similar to ours but in which no activating effect by ascorbic acid was observed. Dr King very kindly informed us of his inability to repeat our experiments and has graciously permitted us to report our findings as to the cause of the discrepancy. We are now able to explain why we obtained the results which we reported previously. In the experiments of our previous paper, we allowed serum phosphatase, β -glycerophosphate, ascorbic acid and buffer (pH 8.9), to incubate for 1 hr. at 37°. At the end of this period, trichloroacetic acid was added to stop enzymic activity. After 30 min. the precipitate was filtered. The solutions were allowed to stand overnight before the determination of phosphate was carried out. King & Delory's experiments differed from ours in that they determined the phosphate immediately after addition of the trichloroacetic acid and the removal of the precipitate. We believed that our procedure was justifiable because we had obtained no appreciable acid hydrolysis of β -glycerophosphate over a 48-hr. period with trichloroacetic or mineral acids at pH 1, in the absence of ascorbic acid (Table I). It is to be regretted that we did not run a control experiment comparing the splitting of phosphate in the presence of ascorbic acid after the 24-hr. period, for to our great surprise we have found that β -glycerophosphate is hydrolysed directly by ascorbic acid at

Table I. *The effect of different acids on β -glycerophosphate*

Temperature, 37°; 50 mg. β -glycerophosphate; 5 mg. ascorbic acid; pH determined electrometrically; total volume, 20 ml.

	Time (hr.)	pH	No ascorbic acid mg. P	Ascorbic acid, 5 mg. mg. P
β -Glycerophosphate + trichloro- acetic acid (0.15 N)	24	1.1	0.0027	0.095
	48	1.1	0.0030	0.118
β -Glycerophosphate + acetic acid (10 N)	24	1.1	0.0035	0.105
	48	1.1	0.0035	0.135
β -Glycerophosphate + hydro- choric acid (0.1 N)	24	1.1	0.0035	0.004
	48	1.1	0.0038	0.005
β -Glycerophosphate + sulphuric acid (0.1 N)	24	1.1	0.0029	0.044
	48	1.1	0.0040	0.086

acid pH, the amount of splitting increasing with time. As Table I shows, ascorbic acid at pH 1 increases markedly the amount of phosphate split from β -glycerophosphate. This effect seems to be equally great with both trichloroacetic and acetic acids. It is considerably less with sulphuric acid, and seems to occur

hardly at all with hydrochloric acid. This direct action of ascorbic acid on β -glycerophosphate was not known and therefore in our published experiments an increased splitting of this substrate, in the presence of serum phosphatase and vitamin, was interpreted as an activation of the enzyme by the vitamin. The theory of ascorbic acid activation of serum phosphatase, advanced in our previous paper, must be abandoned, because it is now evident that the increased phosphorus values reported were brought about by the direct action of ascorbic acid on β -glycerophosphate in acid medium.

The suggestion that increased colour in our colorimetric determination might be due to some other factor than an increased amount of free phosphate led us to perform gravimetric determinations for free phosphate, using ten times as much material as that used in colorimetric work. The results were in fair agreement with those obtained colorimetrically.

In our previous paper it is mentioned that ascorbic acid fails to activate lecithin, sphingomyelin or adenosinephosphoric acid, which in the light of our present knowledge indicates that vitamin C is unable to act directly on these substrates. This observation was extended to include monophenylphosphate. We do not desire to put forth at this time a definite explanation as to why ascorbic acid splits β -glycerophosphate and fails to act on the other substrates investigated. To us it seems possible, however, that ascorbic acid, characterized by its high redox potential, affects the glycerol portion of the phosphoric ester and in this way may bring about splitting of the ester compound.

With regard to the theory advanced by us that increased values in such conditions as Paget's disease and obstructive jaundice are due to an activated phosphatase rather than to an increased amount of the enzyme, it may be mentioned that we have confirmed this theory by other observations made on serum with normal, and serum of high phosphatase activity, results of which will be published shortly.

In Table I it can be seen that no appreciable splitting of the phosphoric ester takes place in the presence of the acids themselves. Even after a hydrolysis period of 3 weeks this was still found to be the case. When ascorbic acid was added to these solutions containing acid and phosphoric ester its ability to split the ester was greatly influenced by the particular acid present. Hydrochloric acid completely overcomes the action of ascorbic acid, while sulphuric acid interferes to a great degree, and even trichloroacetic acid to a lesser degree, acetic acid having the least influence on the action of the vitamin.

Table II contains the results obtained when the concentrations of materials were increased ten times. The diminishing effect of trichloroacetic acid is evident.

Table II. *The effect of ascorbic acid on β -glycerophosphate using increased concentrations (ten times usual concentration)*

Temperature, 37°; 500 mg. β -glycerophosphate; 50 mg. ascorbic acid; 10 ml. 10% trichloroacetic acid; total volume 20 ml.

- (a) β -Glycerophosphate + trichloroacetic acid pH = 1.1.
 (b) β -Glycerophosphate + trichloroacetic acid + ascorbic acid pH = 1.1.
 (c) β -Glycerophosphate + ascorbic acid pH = 3.1-6.9.

Time in thermostat days	a mg. P	b mg. P	c mg. P
2	0.003	0.250	0.570
10	0.006	0.500	1.550
20	0.011	1.250	1.675

SUMMARY

1. The direct action of ascorbic acid alone on sodium β -glycerophosphate is reported.
2. The failure of hydrochloric, sulphuric, trichloroacetic, and acetic acids to hydrolyse β -glycerophosphate at $pH=1$ has been shown.
3. The influence of hydrochloric, sulphuric, trichloroacetic and acetic acids, on the action of ascorbic acid, with regard to its splitting of β -glycerophosphate, is demonstrated.
4. The theory of ascorbic acid activation of serum phosphatase, outlined in a previous paper, is abandoned in the light of the present knowledge of ascorbic acid action on sodium β -glycerophosphate.

REFERENCES

- King & Delory (1938). *Biochem. J.* **32**, 1157.
Thannhauser, Reichel & Grattan (1937). *J. biol. Chem.* **121**, 697.