LVIII. NOTE ON AN ACCELERATING ACTION OF URIC ACID ON THE XANTHINE OXIDASE SYSTEM.

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IN studying the effect of the xanthine oxidase system on other oxidising systems, it was desired to use some specific inhibitor of this oxidase in order to dissociate the effects produced by the oxidase from possible effects produced by other substances present in the enzyme solution. Dixon and Thurlow [1924] showed that uric acid retards the oxidation of hypoxanthine by xanthine oxidase in presence of methylene blue, and this acid therefore seemed to be a suitable inhibitor to use. On testing the effect of uric acid on the reduction of methylene blue by hypoxanthine and xanthine oxidase, however, it was found that the uric acid produced no inhibition but, instead, a marked acceleration of the reaction. At first, it appeared that some oxidisable impurity was present in the uric acid, but this possibility was excluded when it was found that other specimens of pure uric acid produced the same accelerating effect. At first sight, this result is directly contradictory to the results obtained by Dixon and Thurlow. On further consideration of the curves given in their paper, however, it appeared that an acceleration by uric acid of the activity of xanthine oxidase was not incompatible with the uric acid inhibitions which they described. They showed that with a constant amount of xanthine oxidase, on increasing the amount of hypoxanthine the rate of reduction of methylene blue remained constant until a certain critical concentration was reached, after which increasing the hypoxanthine concentration produced a marked and progressive inhibition in the rate of oxidation. They explained this inhibition as being due to the blocking of the enzyme surface by the excess of hypoxanthine, whereby the access of methylene blue to the surface is hindered and the reaction is retarded. By keeping the hypoxanthine concentration constant and adding increasing amounts of uric acid, Dixon and Thurlow found a similar increasing inhibition of the reaction, the inhibition in this case being ascribed to adsorption of uric acid on the enzyme surface preventing free access of methylene blue.

Assuming this explanation to be correct, it appears that the accelerating action of uric acid which I have observed can be explained in the following way. Let us consider a concentration of hypoxanthine sufficiently high to produce, with a given amount of enzyme, a considerable inhibition in its own oxidation.

On adding uric acid, part of the hypoxanthine adsorbed on the enzyme surface will be driven off and replaced by uric acid. That is, some of the one inhibitor, hypoxanthine, will be replaced by the other inhibitor, uric acid. If uric acid be a less powerful inhibitor of the reaction than hypoxanthine—and the inhibition-concentration curves of the two purines indicate that this is the case—then the result of adding uric acid should be the replacing of one inhibitor by a less powerful inhibitor, and a relative acceleration should be observed.

If this explanation be the true one, it would be expected that by keeping the enzyme constant and increasing the hypoxanthine to a concentration at which it produced an inhibition, the addition of uric acid would produce an inhibition which would be more than counteracted by its removing the more powerful inhibitor, hypoxanthine. That is, by increasing the concentration of hypoxanthine and keeping everything else constant, an inhibition by uric acid should be changed into an acceleration. That this actually occurs is shown by the following experiment.

Exp. 1. Thunberg vacuum tubes were used, and after filling, the tubes were evacuated, filled with nitrogen, re-evacuated and put in a bath at 37°. In all experiments described in this paper, 0.5 cc. of methylene blue (1 part in 5000 parts of water) was used in each tube, and the total volume of solution in the tubes was always 2.5 cc., this volume being made up where necessary by addition of the requisite amount of distilled water. The xanthine oxidase was the "whey preparation" described by Dixon and Kodama [1926], prepared by coagulating milk with rennin and half-saturating the whey with ammonium sulphate. 200 mg. of the dry preparation were dissolved in 5 cc. phosphate buffer and brought to p_H 7.4 with dilute NaOH. Both the hypoxanthine and the uric acid solutions were made by dissolving in slight excess of NaOH and bringing to p_H 7.4 with dilute HCl immediately before using. The hypoxanthine solution contained 1 mg. per cc. and the uric acid solution 2.5 mg. per cc. 0.2 cc. xanthine oxidase was used in each tube in this experiment.

Substances added	Time in mins. for M.B. reduction	Uric acid effect
1. 0.1 cc. hypoxanthine	7	
2. 0.1 cc. hypoxanthine + 1 cc. uric acid	14½	Inhibition
3. 0.4 cc. hypoxanthine	15½	
4. 0.4 cc. hypoxanthine + 1 cc. uric acid	11½	Acceleration
5. 0.8 cc. hypoxanthine	33½	
6. 0.8 cc. hypoxanthine + 1 cc. uric acid	14½	Acceleration

Comparing tubes 1 and 2 with tubes 3 and 4 or with 5 and 6, it is clear that, by increasing the hypoxanthine concentration 4 times or 8 times, the inhibitory effect of uric acid is changed into a marked acceleration. This experiment and the others described in this paper have been repeated several times with the same results.

Dixon and Thurlow showed that the critical concentration of hypoxanthine at which it commences to inhibit its own oxidation is dependent on the enzyme concentration: the more concentrated the enzyme solution, the

more hypoxanthine is necessary before the critical concentration is reached. By keeping the hypoxanthine concentration constant and decreasing the enzyme concentration, the hypoxanthine would become an inhibitor, and it might be expected therefore that under these conditions the inhibition by uric acid would again be changed into an acceleration. Exp. 2 shows that this actually occurs.

Exp. 2. The enzyme solution and the purine solutions were the same as those used in the previous experiment. 0.2 cc. of hypoxanthine was used in each tube. 0.3 cc. phosphate buffer was added to tubes 3 and 4 to keep the phosphate concentration the same in all the solutions.

Substances added	Time in mins. for reduction of M.B.	Uric acid effect
1. 0.4 cc. enzyme	5½	
2. 0.4 cc. enzyme + 1 cc. uric acid	10½	Inhibition
3. 0.1 cc. enzyme	46	
4. 0.1 cc. enzyme + 1 cc. uric acid	24½	Acceleration

It can be seen that, with the hypoxanthine kept constant, the reduction of the enzyme concentration to one-quarter has changed an inhibition by uric acid into a marked acceleration.

It is clear, therefore, that an inhibition by uric acid can be changed into an acceleration either by increasing the concentration of hypoxanthine or by decreasing the enzyme concentration. Whether an inhibition or acceleration is produced under given conditions by uric acid depends on the relationship between the concentrations of hypoxanthine and enzyme.

In Exp. 3 a similar effect was obtained, using fresh milk to supply xanthine oxidase.

Exp. 3. Each tube contained 0.25 cc. of fresh cow's milk. The hypoxanthine solution used in this experiment contained 10 mg. in 3 cc., the uric acid concentration being the same as in the previous experiments.

Substances added	Time in mins. for M.B. reduction	Uric acid effect
1. 0.03 cc. hypoxanthine	5½	
2. 0.03 cc. hypoxanthine + 1 cc. uric acid	9½	Inhibition
3. 0.6 cc. hypoxanthine	13	
4. 0.6 cc. hypoxanthine + 1 cc. uric acid	10½	Acceleration
5. None	∞	

It can be seen that, using milk, the inhibition and acceleration produced by uric acid are much smaller than those observed when purified xanthine oxidase is used. Using milk, it is necessary to increase the hypoxanthine concentration to a much higher figure in order to obtain an acceleration by uric acid. Both the hypoxanthine and uric acid appear to be adsorbed on the enzyme to a much smaller extent in milk, probably owing to the adsorption by the enzyme of other substances present in milk.

It would naturally be expected that, in the transition from an inhibition to an acceleration by uric acid, an intermediate stage would be reached at

which the addition of uric acid produced no effect on the reaction. This condition has been realised experimentally on more than one occasion.

SUMMARY.

In the oxidation of hypoxanthine in presence of xanthine oxidase and methylene blue, the addition of uric acid may under suitable conditions produce a marked acceleration instead of an inhibition. The inhibiting action of uric acid on the reaction may be converted into an acceleration either by increasing the hypoxanthine or decreasing the enzyme concentration.

The accelerating effect appears to be due to the replacement on the enzyme surface of the inhibitor hypoxanthine by the less powerful inhibitor uric acid.

REFERENCES.

- Dixon and Thurlow (1924). *Biochem. J.* **18**, 980.
—— and Kodama (1926). *Biochem. J.* **20**, 1105.