15059

Studies On the Inhibition of Xanthine Oxidase.*

SEYMOUR J. GRAY AND ROSE Z. FELSHER.

From the Department of Medicine, University of Chicago.

The formation of uric acid from xanthine and hypoxanthine depends upon the presence of xanthine oxidase. The oxidized form of *p*-aminophenol is a powerful inhibitor of xanthine oxidase activity. This has been demonstrated by Bernheim and coworkers[1] in studies with liver xanthine oxidase and by Blauch, Koch, and Hanke[2] in their work with rat blood xanthine oxidase. Since this inhibition occurs with very low concentrations of *p*-aminophenol, its effect on the uric acid production of a patient with gout was studied. Further studies, *in vitro,* of other substances which might possibly inhibit xanthine oxidase were also made.

Methods. The *p*-aminophenol was dissolved in concentrations of 0.5 to 4.0 g in 150-350 cc .1M phosphate buffer at pH 7.3 and warmed until a brown color developed. The solution was then allowed to stand exposed to the air for one hour until a brown suspension appeared. Increasing amounts of *p*-aminophenol from 0.5 g to 4.0 g were injected intravenously daily or on alternate days into a patient with very severe gout. The blood and urine uric acid was determined by the direct method of Folin.[3] The patient was maintained on a purine-free diet.

The inhibitory effect of free iodine dissolved in potassium or sodium iodide solution, on milk xanthine oxidase, was determined manometrically in the Warburg apparatus at 38°, at pH 7.3, approximating that of blood. Many other substances, including pyrogallol, were likewise tested for their inhibitory effect. The xanthine oxidase was prepared essentially by the method of Ball.[4] The enzyme was somewhat less pure than that of Ball.

Results and Discussion. At pH 7.3 a 7 x 10^{-3} M solution of *p*-aminophenol caused a 77% inhibition of milk xanthine oxidase.[4] The actual inhibition may have been considerably higher, however, since Bernheim[1] demonstrated that milk contains a substance which inactivates the oxidized form of *p*-aminophenol.

The intravenous administration of 0.5 to 4.0 g of the *p*-aminophenol did not affect the uric acid output in the urine or the blood uric acid level. During 10 days of injection the average of the daily urinary uric acid output was 405 mg, compared with corresponding values of 390 and 391 mg, for weekly periods, before and after injections, respectively. The blood uric acid remained essentially unchanged: 7.0 mg % before injection, 7.3 mg % during the injections, and a week later 6.6 mg %.

When *p*-aminophenol solutions are warmed or exposed to the air a brown color develops, indicating, according to Bernheim,[1] that the substance is partly oxidized to quinimine; the reduced and oxidized forms are thus in equilibrium, forming a reversible oxidation-reduction system.[1] Bernheim's studies indicated that the inhibition was due exclusively to the oxidized form of the drug. The negative results of intravenous injection in the human may be explained by the conversion of *p*-aminophenol to the reduced inactive form. The freshly excreted urine was clear amber in color and turned brown only upon shaking or warming, suggesting that the *p*-aminophenol

* This study was aided by a grant from the Nutrition Research Laboratories.

The authors wish to express their appreciation to Dr. E. S. G. Barron for his valuable advice and the use of his laboratory facilities.

1 Bernheim, F., Bernheim, M. L. C., and Michael, H., *J. Pharm. and Exp. Ther.,* 1937, **61**, 311; Bernheim, F., and Bernheim, M. L. C., *J. Biol. Chem.,* 1938, **123**, 307.

2 Blauch, M. B., Koch, F. C., and Hanke, M. E., *J. Biol. Chem.,* 1939, **130**, 471.

3 Folin, O., *J. Biol. Chem.,* 1933, **101**, 111; *ibid.,* 1934, **106**, 311.

4 Ball, E. G., *J. Biol. Chem.,* 1939, **128**, 51.

TABLE I.

Effect of Iodine on Xanthine Oxidase.

0.3 cc of inhibitor; 0.1 M phosphate buffer, pH 7.3; substrate, 0.2 cc of 5 × 10^{-2} M xanthine; enzyme and water to 3.0 cc; 38°C; duration of experiment, 30 minutes.

Inhibitor	O_2 consumption (blank subtracted) cmm	Inhibition, %
None	122.0	
.008 M Iodine + .024 M Potassium Iodide	16.9	83.8
.006 " " + .016 " " "	24.0	79.8
.004 " " + .012 " " "	69.3	43.2
.002 " " + .006 " " "	99.0	18.9
None	122.0	
.008 M Iodine + .026 M Sodium Iodide	2.3	98.2
.006 " " + .017 " " "	5.4	95.6
.004 " " + .013 " " "	61.3	49.8
None	58.5	
0.1 M Sodium Iodide	62.3	0
None	70.7	
0.1 M Sodium Iodide	71.9	0

was excreted in the reduced form.

The effectiveness of the drug depended also upon its diffusibility into the intact tissue cells and upon the hydrogen ion concentration. Bernheim's experiments indicated that the inhibitory effect of *p*-aminophenol was more uniform when macerated cells were used rather than tissue slices. The greatest inhibition occurred, moreover, at pH 6.4 to 6.7 and decreased considerably at the pH of the blood. These factors may be responsible for the ineffectiveness of *p*-aminophenol when injected into the human. They may also explain the relatively few toxic symptoms noted, consisting only of a transient elevation of the temperature upon 2 occasions to 100.2° and 101°, and slight nausea on one occasion.

Of a large number of substances tested manometrically for their inhibitory effect upon milk xanthine oxidase, only 2 demonstrated some inhibition. Dilutions of Lugol's solution (10% potassium iodide and 5% iodine) gave 79.8 and 83.8% inhibition with iodine concentrations of 0.006 and 0.008 M, respectively (Table I). At these concentrations of iodine in sodium iodide solution somewhat greater inhibitions (95.6 to 98.2%) were obtained, but in .002 and .004 M solutions, the inhibition was considerably reduced. Sodium iodide alone in 0.1 M solution, produced no inhibition whatever, indicating that the free iodine was the effective inhibitor of xanthine oxidase. This effect was not specific for xanthine oxidase since same concentrations of iodine inhibited other enzyme systems like uricase.

TABLE II.

Effect of Pyrogallol on Milk Xanthine Oxidase.

0.3 cc of inhibitor; 0.1 M phosphate buffer, pH 7.3; substrate, 0.2 cc of 5 × 10^{-2} M xanthine; enzyme and water to 3.0 cc; 38°C; duration of experiment 30 minutes.

Inhibitor	O_2 consumption (blank subtracted) cmm	Inhibition, %
None	127.3	
0.01 M Pyrogallol	45.3	64.8
None	134.3	
0.01 " "	42.9	68.0
0.001 " "	74.8	44.3
None	72.2	
0.01 " "	24.2	67.0
0.001 " "	39.8	44.9

0.001 M pyrogallol inhibited xanthine oxidase approximately 44%, a .01 M solution 65% (Table II). These concentrations of pyrogallol had no effect on partially purified uricase, but this substance is exceedingly toxic and has no clinical value.

Many other substances were tested in concentrations of 0.001 M or higher and caused no appreciable inhibition of the milk xanthine oxidase. Among the substances tested manometrically were *p*-nitrophenol, resorcinol, catechol, alpha-naphthol, *p*-bromphenol, phloroglucinol, anthraquinone, tyrosine, tryptophane, *p*-toluidine, cysteine, glycine, liver extract, alloxan, thiouracil, quinidine, neoarsphenamine, colchicine, quinine, thyroxine, and urea.

Summary. The repeated intravenous administration of increasing doses 0.5 to 4.0 g of *p*-aminophenol to a patient with gout did not affect the uric acid output in the urine or the blood uric acid level; occasionally there was transient elevation of temperature and nausea. Free iodine, in .008 M solution, caused an 88% inhibition of milk xanthine oxidase but was not specific for this enzyme. 0.001 M pyrogallol inhibited xanthine oxidase approximately 44% and had no effect upon other enzymes like uricase. Many other substances were tested and found to cause no appreciable inhibition of xanthine oxidase.

15060 P

Acute and Subacute Toxicity of Pure Citrinin.

A. M. AMBROSE AND FLOYD DEEDS.

From the Pharmacological Laboratory, Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture, Albany, Calif.

Citrinin is an antibiotic produced by *Penicillium citrinum*,[1] and by *Aspergillus sp.* of the *candidus* group.[2] Robinson[3] has reported on the acute toxicity of citrinin in mice following oral and intraperitoneal administration. Timonin and Rouatt[4] have reported toxicity data obtained by Noble, who administered citrinin intraperitoneally and subcutaneously to mice, intraperitoneally to rats, and orally and subcutaneously to rabbits. In the following summary the data of these investigations have been recalculated in terms of milligrams per kilogram of body weight so as to make them directly comparable with the data presented in this report.

Because of the low solubility of citrinin in water, Robinson[3] administered it to mice as a suspension in 10% gum acacia solution. In a series of 30 mice he gave the suspended citrinin orally in doses ranging from 12.5 to 500 mg per kilo of body weight. Doses of 100 mg or less produced no fatalities, but 200 and 500 mg killed 40 and 100% of the mice, respectively, in 7 days. In another series of 30 mice he found 20% and 100% mortality resulting from intraperitoneal administration of 200 and 400 mg, respectively.

In the toxicity tests reported by Timonin and Rouatt[4] the citrinin was dissolved in sodium hydroxide (pH 8-9.5) and the solution adjusted to a neutral reaction as rapidly as possible with hydrochloric acid. A series of 20 mice were given citrinin intraperitoneally in doses ranging from 25 to 125 mg per kilo of body weight. All mice receiving 100 mg died in 6 to 18 hours, and all mice receiving 125 mg died in 5 to 6 hours. None of the mice given 50 mg died after a single administration, but repetition of this dose 24 hours later produced 40% mortality. In a series of mice receiving 100 mg subcutaneously, 80% mortality resulted in 4 to 24 hours. No deaths occurred in 5 rats given 50 mg per kilo intraperitoneally. One rabbit receiving 25.7 mg per kilo in 2 doses by mouth and a second rabbit given 30 mg (absolute dose) subcutaneously daily for 5 days showed no ill effects.

Unless adequate precautions are taken, citrinin is apt to be contaminated with oxidation products and other impurities derived from the culture medium. Even pure citrinin is contaminated readily with oxidation products when it is dissolved in alcohol or in aqueous solution on the alkaline side of neutrality. The high degree of purity of the citrinin used in obtaining the following toxic-

[1] Hetherington, A. C., and Raistrick, H., *Philosophical Transactions*, 1931, **220**, 269.

[2] Timonin, M. I., and Rouatt, J. W., *Canadian J. Public Health*, 1944, **35**, 80.

[3] Robinson, H. J., A Thesis submitted to the Graduate Faculty of Rutgers University, New Brunswick, N.J., May, 1943.

[4] Timonin, M. I., and Rouatt, J. W., *Canadian J. Public Health*, 1944, **35**, 396.