4a,12a-Anhydrodedimethylaminotetracycline (V).—12a-Deoxytetracycline (50 mg.) was dissolved in 20 cc. of dimethoxyethane and 1.25 cc. of a chloroform solution of perbenzoic acid (12.9 mg./cc.) was added. The stoppered reaction solution was left to stand overnight at room temperature. The solvent was evaporated to about 1 cc. and the brown-red crystals which formed were filtered, washed with ether and dried at 60° *in vacuo;* wt. 8.48 mg., m.p. dec. slowly above 250°. This compound was identical by all criteria with that described in the preceding paper.[7a]

Anhydro-12a-deoxytetracycline Hydrobromide (IV).—Eight grams of 12a-deoxytetracycline was dissolved in 350 cc. of glacial acetic acid at 70° and the solution was treated with charcoal and filtered. To the filtrate, reheated to 70° was added 16 cc. of 31% HBr in glacial acetic acid. The color changed from yellow-orange to red, and within a few seconds the product deposited as light yellow flocculent crystals. The mixture was heated to 80° during which time the crystals changed to a more dense red colored solid. After heating for 15 minutes the mixture was cooled to room temperature, and the product was filtered, washed with acetic acid, chloroform, ether and dried at 60° in vacuum; wt. 7.46 g., m.p. 252° dec. The ultraviolet absorption spectra show $\lambda_{max}^{0.1 N\ HCl}$ 270 mμ, log ε 4.542; 425 mμ, log ε 3.922; $\lambda_{max}^{0.1 M\ Na_2B_4O_7}$ 268 mμ, log ε 4.572; 425 mμ, log ε 4.062.

Anal. Calcd. for $C_{22}H_{23}N_2O_6Br$: C, 53.8; H, 4.73; N, 5.72; Br, 16.32. Found: C, 53.15; H, 5.49; N, 5.29; Br, 17.22.

[CONTRIBUTION FROM THE DEPARTMENTS OF MICROBIOLOGY AND NEUROLOGY, COLLEGE OF PHYSICIANS AND SURGEONS, COLUMBIA UNIVERSITY, AND THE NEUROLOGICAL INSTITUTE, PRESBYTERIAN HOSPITAL, NEW YORK]

Immunochemical Studies on Blood Groups.[1] XXV. The Action of Coffee Bean α-Galactosidase on Blood Group B and BP1 Substances

BY MARIE LUISE ZARNITZ[2] AND ELVIN A. KABAT

RECEIVED JANUARY 29, 1960

Coffee bean α-galactosidase splits galactose from the blood group B and BP1 substances. The precipitating power of the B substance for human anti-B was reduced but its capacity to inhibit hemagglutination of B cells by anti-B was completely destroyed as was the precipitability of BP1 for human anti-BP1. Cross reactivity of both enzyme-treated substances with Type XIV horse antipneumococcal sera was increased. The implications of these findings for the structure of the B and BP1 substances are discussed.

Studies in several laboratories have established that the specificity of the blood group A, B and O(H) substances is associated with various oligosaccharide side chains which are split off by mild acid hydrolysis.[3-6] In the case of the blood group B substance, the specific side chains have a terminal, non-reducing galactose linked α- to the next residue. The evidence for this rests primarily on the observation that oligosaccharides and other galactosides having a terminal non-reducing galactose were more effective in inhibiting B-anti-B precipitation or the hemagglutination of B cells by anti-B than was galactose and that β-linked galactosides were less effective. No inhibition was observed with the other sugars present in the blood group substances.[7] Morgan[8] recently has reported that galactosyl-1,3-α-galactose was a better inhibitor of B-anti-B hemagglutination than was melibiose; galactose was not active under the conditions used. In addition, the action of an enzyme from *Trichomonas foetus* in destroying the activity of soluble blood group B substance was inhibited[9] by galactose, by methyl-α and β-D-galactopyranosides and by lactose and melibiose but not by other sugars and glycosides. Treatment of B substance with *T. foetus* enzyme split off galactose with the development of O(H) specificity[10a] and Iseki[10b] has obtained an enzyme from a strain named *Clostridium maebashi* which splits galactose from blood group B substance.

Another kind of terminal α-galactose may be exposed by mild acid hydrolysis of blood group B substance.[11] This procedure has been shown to liberate fucose and various oligosaccharides and, depending upon conditions, to destroy blood group specificity partially or completely. The non-dialyzable residue (BP1) from a B substance treated in this manner gave rise to antibodies on injection into a human of blood group A_1B. Precipitation of the BP1 substance by its antibody showed a specificity for α-galactosyl residues. Evidence that the α-galactosyl residues involved in B and BP1 specificity were linked differently to the second sugar unit was obtained.[11] The independence of the B, the BP1 grouping and the terminal β-linked galactoses responsible[12-14] for cross reactivity with Type XIV antipneumococcal sera could also be shown by time–hydrolysis curves and assaying for all three activities.[14]

Additional information about the B and BP1 reactive groupings could be obtained, were it possible to split off the terminal α-galactosyl residues and ascertain the effect on the various immunochemical properties. An α-galactosidase from coffee beans was described by Helferich and Vorsatz[15]

(1) Aided by grants from the National Science Foundation (G-5208) and the William J. Matheson Commission.

(2) Rockefeller Foundation Fellow 1958–1959.

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and more recently by Courtois and co-workers.[16-18] The latter group showed that the enzyme acted upon polysaccharides containing terminal α-galactosyl residues and would also effect transglycosylation with the synthesis of new oligosaccharides containing terminal non-reducing galactoses. The present studies demonstrate the liberation of galactose from both the B and the BP1 substances by this enzyme. With B substance this resulted in the complete loss of B activity as assayed by hemagglutination inhibition and partial loss of precipitability with human anti-B. With BP1 substance, precipitability with anti-B was eliminated. Both enzyme-treated materials showed increased cross-reactivity with Type XIV anti-pneumococcal serum, but the reaction of the original B and enzyme-treated B substances was very small.

Experimental

Materials and Methods.—Blood group B and BP1 substances (Beach), prepared by Dr. Sidney Leskowitz and described in previous publications[11,19] were dissolved and dialyzed against 0.1 *M* acetate buffer *p*H 4.9 for 2 days at 0° using continuous flow of buffer in the presence of toluene. The Beach P1 had BP1 as well as some B activity, the latter not having been completely destroyed under the condition of hydrolysis used.[11]

A sample of α-galactosidase from coffee beans (Santos) was kindly supplied by Dr. J. E. Courtois, Laboratoire de Chimie Biologie, Faculté de Pharmacie, Paris. As described,[20] 50 mg. was suspended in 10 ml. of H_2O, the mixture rocked for 1 hour at room temperature and then centrifuged at 0°. The supernatant was dialyzed against 5 changes H_2O at 0° (toluene added), the non-dialyzable portion clarified by centrifugation in the cold and the supernatant kept at 0°. Nitrogen in the enzyme preparation was determined by Kjeldahl. All enzyme experiments were carried out at *p*H 4.9 and 37° in 0.05 *M* acetate buffer. The specificity of the enzyme preparation was checked by reducing sugar determination and paper chromatography with melibiose and lactose as substrates; 100 μg. melibiose in 0.9 ml. buffer was hydrolyzed within 16 hours by 0.1 ml. enzyme solution (196 μg. N/ml.), while with lactose under the same conditions no formation of monosaccharides could be detected.

For the separation of the sugars liberated by the enzyme from the blood group substances a charcoal-Celite column, as described by Whistler and Durso[21] and modified,[22] was employed using a mixture of 10 g. Darco G-60 and 10 g. of Celite No. 535. Samples of 5 ml. were collected in an automatic fraction collector and the effluent was analyzed as described previously[22] for reducing sugar, galactose, methylpentose and hexosamine.

Blood group B and BP1 substances and their enzymatic degradation products were estimated by the inhibition of hemagglutination and by quantitative precipitin assays.[7,23] With anti-B and anti-BP1 the antibody nitrogen was determined by the Folin–Ciocalteu tyrosine method.[23,24] In the cross reactions with horse anti-S XIV the precipitates were analyzed for antibody nitrogen by the Markham micro-Kjeldahl method.[23] These various sera were employed: anti-B serum 310_3 and 310_4,[11] anti-BP1 serum 262_{4-6} absorbed with B substance (PM phenol-insoluble).[11] Type XIV anti-pneumococcal horse serum H 635 bled 11-25-38 was obtained from the New York State Department of Health Laboratories through the courtesy of Dr. J. L. Hendry.

Procedure.—Preliminary experiments showed that a 1:10 ratio in weight of enzyme protein to substrate is necessary for sufficient splitting. No increase in reducing sugar was obtained in 24 hours at 37° either with enzyme in the absence of blood group substance or with blood group substance plus enzyme which had been heated at 100° for 15 minutes. The immunochemical behavior in the latter instance was not changed in hemagglutination inhibition (Table I) or in precipitability with human anti-B. To 15 ml. of substrate solution containing 102 mg. of Beach or 104 mg. Beach P1 respectively in 0.1 *M* acetate buffer *p*H 4.9, 15 ml. of α-galactosidase solution containing 14 mg. of protein and a few drops of toluene were added, and the mixture incubated at 37° for four days. Samples of 350 μg. blood group substance were taken out at various times, heated at 100°, analyzed for reducing sugar and tested for their ability to inhibit the hemagglutination of B cells by anti-B. After 4 days the reaction mixture was heated for 20 minutes in a boiling water bath and dialyzed against 5 changes of water in the presence of a few drops of chloroform. The combined dialysates were concentrated to 20 ml. under reduced pressure at 40° and chromatographed on the charcoal–Celite column, and then eluting with water an ethanol gradient. The non-dialyzable portion was lyophilized and dried over P_2O_5, yielding 94.4 mg. of Beach E.T. (enzyme-treated) and 95.5 mg. of Beach P1 E.T. Sixty-nine mg. of Beach E.T. was extracted three times with 90% phenol. The residue was washed with ethanol and ether and dried *in vacuo* yielding 48.8 mg. of Beach E.T. phenol insoluble. From the combined phenol extracts 8.2 mg. could ever recovered by addition of ethanol up to 20% by volume (Beach E.T; 20% ppt.). 76 mg. of Beach P1 E.T. was dissolved in phenol and by addition of ethanol to this solution fractions precipitating between 0–10% and 10–20% ethanol were obtained. After washing with ethanol and ether and drying *in vacuo* 38.0 mg. of Beach P1 E.T. 10% and 20.8 mg. of Beach P1 E.T. 20% precipitates were recovered. All substances were soluble in saline.

Results

Incubation of an α-galactosidase preparation from coffee beans with human ovarian cyst B blood group substance or its corresponding P1 fraction leads to an increase in reducing sugar while the ability of these substances to inhibit the hemagglutination disappears (Table I). The dialyzable split products were fractionated on a charcoal–Celite column and analyzed for reducing sugar, galactose, methylpentose, hexosamine and N-acetylhexosamine. In the case of the B substance the dialysate from 102 mg. blood group substance contained 5.21 mg. of reducing sugar as glucose as compared with an increase of 4.6 mg. found by assay during enzyme action (Table I). Figure 1 (upper chromatogram) shows that the main split product is galactose which was eluted with water. 4.45 mg. of reducing sugar was in the galactose peak of the 4.95 mg. placed on the column. Between 0 and 2% ethanol, traces of methylpentose (25 μg.) were eluted. 250 ml. fractions of the effluent in which no peaks were observed were pooled and analyzed for their content of any of the four possible sugars but none was detected in significant amounts. Similar results were obtained in the dialysate from α-galactosidase treated Beach P1. From 104 mg. of Beach P1, 6.44 mg. of reducing sugar resulted as dialyzable material. The chromatogram (Fig. 1 lower curve) with 6.1 mg. of reducing sugar shows the main galactose peak (5.1 mg. as reducing sugar) and 4 very small peaks. In the pooled and concentrated fractions, the sugar appearing between 1 and 2% ethanol could be

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TABLE I

ACTION OF COFFEE BEAN α-GALACTOSIDASE ON BLOOD GROUP B AND BP1 SUBSTANCES

	Incubation time, days	Red sugar, mg.	Hemagglutination inhibition[a] Amount blood group substance in μg.									
			100	50	25	10	5	2.5	1.0	0.5	0.25	0.1
Beach (102 mg.) + enzyme	0	16.0	−	−	−	−	−	−	−	++	++++	++++
	1	19.0	−	−	+±	++	+++	++++	++++	++++	++++	++++
	2	19.9	−	−	++	+++±	++++	++++	++++	++++	++++	++++
	3	20.6	++±	++±	+±	+++±	++++	+++±	++++	++++	++++	++++
	4	20.6	+±	+++±	+++	+++±	++++	++++	++++	++++	++++	++++
Beach P1 (2.9 mg.)	1	0.49	−	−	−	±	+	+++	++++	++++	++++	++++
Beach P1 (2.9 mg.) + heated enzyme	1	0.65	−	−	−	+±	+++	+++±	++++	++++	++++	++++
Beach P1 (2.9 mg.) + active enzyme	1	0.84	+++±	+++±	++++	++++	++++	++++	++++	++++	++++	++++

[a] Procedure for hemagglutination inhibition: 0.1 ml. of anti-B serum 310_3 (diluted 1:15) was incubated with 0.8 ml. of saline containing different amounts of blood group substances for 30 minutes at 37°. 0.1 ml. of 4% washed human B erythrocytes were added, incubated at 37° for 1 hour and the degree of hemagglutination (− to ++++) read after light centrifugation.

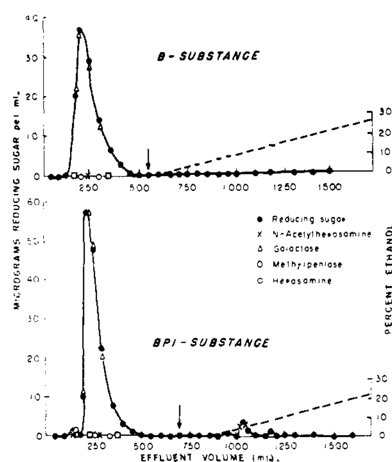

Fig. 1.—Dialyzable sugars liberated by coffee bean α-galactosidase from blood group B and BP1 substances. The graphs represent the chromatographic patterns of Beach e.t. (upper graph) and Beach P1 e.t. (lower graph). The arrow shows the point at which the ethanol gradient was imposed: the dashed lines represent the ethanol concentration in the effluent.

identified as fucose (24 μg.) and the peak between 5 and 10% ethanol as N-acetylglucosamine (102 μg.) while the amounts of the other two were not enough for quantitative analysis.

The fractions obtained by phenol–ethanol from the non-dialyzable residues of the enzyme-treated B and BP1 substances all showed a decrease in capacity to precipitate anti-B (Fig. 2, left and center graphs).

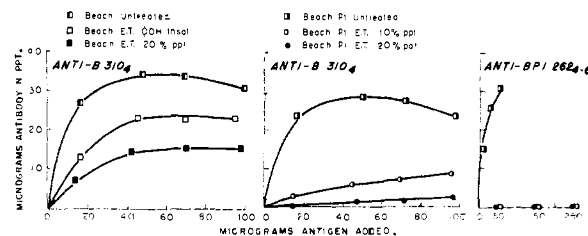

Fig. 2.—Precipitin reactions of untreated and α-galactosidase treated B and BP1 substances with human anti-B and anti-BP1 serum. Left graph: B substances with 1.0 ml. of anti-B serum 310_4; center graph: BP1 substances with 1.0 ml. of anti-B serum 310_4; right graph: BP1 substances with 3.0 ml. of anti-BP1 serum 262_{4-6}.

From the supernatants to which enzyme-treated B or BP1 substances had been added, the remaining antibody could be recovered by addition of untreated B or BP1, respectively. The isolated enzyme-treated B and BP1 fractions also did not inhibit hemagglutination of B cells by anti-B. The isolated enzyme-treated BP1 fractions failed completely to precipitate anti-BP1 (Fig. 2, graph at right), and from the supernatant untreated BP1 precipitated the antibody.

In the cross reaction with Type XIV antipneumococcal sera, the enzyme-treated B and BP1 substances showed an increase in precipitating power (Fig. 3). With the original B substance, the cross reactivity was very low; 500 μg. substance precipitated only about 7 μg. N, and this increased to 12 and 16 μg. N for the enzyme-treated phenol

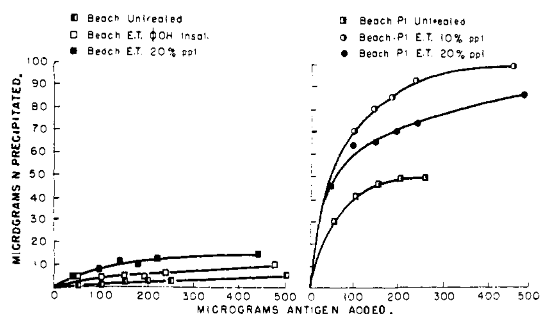

Fig. 3.—Cross reactions of untreated and α-galactosidase treated B and BP1 blood group substances with 0.5 ml. of Type XIV anti-pneumococcal serum H 635, bled 11-25-38. Left graph: B substances; right graph: BP1 substances.

insoluble and 10% precipitate. With the BP1 substance the cross reactivity was initially substantial and increased significantly on enzyme treatment.

Discussion

The demonstration that galactose is split from both the B and BP1 substances by the coffee bean α-galactosidase with loss of their respective activities provides additional evidence that terminal non-reducing α-linked galactoses are responsible for the specificity of each of these materials. This is also consistent with the findings of Courtois, *et al.*,[16] that the enzyme acts on terminal α-linked galactosides having a free primary hydroxyl and splitting them off one at a time. The independence of the terminal α-galactosyl groupings responsible for B and BP1 specificity[7,11] and the terminal β-galactosyl groups determining Type XIV cross reactivity[12–14] of these substances was established previously.[11,14] In addition, it has been shown that both B and BP1 activities were retained after treatment with Clostridial enzymes while cross reactivity with Type XIV antibody was almost completely destroyed.[4] The present findings show that removal of the terminal α-galactoses associated with B and BP1 activity actually results in increased cross reactivity with Type antibody (Fig. 3), although with the B substance the extent of the cross reaction was slight. This increased cross reactivity in the case of the BP1 would indicate that removal of the terminal α-galactosyl unit may leave a terminal β-galactosyl residue which is responsible for the increased cross reactivity and suggests therefore that the BP1 specific grouping may be galactosyl-α-galactosyl-β but could equally well be a chain of several α-linked galactoses which would have been sequentially hydrolyzed by the enzyme until the β-linked galactose became terminal. Another possibility would be that steric factors are preventing the β-galactosyl residues from reacting with Type XIV antibody and that the removal of the α-galactosyl residues permits them to react. With the enzyme-treated B substance the increased cross reactivity is very small and need not be attributed to the splitting of the terminal α-galactosyl involved in B specificity but could also possibly be due to splitting a few BP1 groupings which were not detected in the original B substance.

Of especial interest is the finding that the enzyme-treated B substance retained substantial precipitating power for anti-B and the enzyme-treated BP1 substance retained some ability to precipitate anti-B, although it had completely lost its capacity to precipitate anti-BP1. This would suggest, in terms of findings in the dextran–antidextran system,[25,26] that the specific polysaccharide side chain determining B specificity may be longer than that determining BP1 specificity unless it is ultimately demonstrated that BP1 specificity involves several α-linked galactoses which were split sequentially by the enzyme. That hemagglutination inhibiting potency is completely destroyed but precipitability is only partially reduced is in accord with findings that the terminal non-reducing residue contributes the largest portion to the total free energy of binding in antigen–antibody reactions[25,26] and that inhibition of hemagglutination requires considerably higher concentrations of mono- and oligosaccharides than does inhibition of precipitation.[7]

The nature of the second sugar in the B specific grouping is not clear. Based on quantitative inhibition studies with oligosaccharides it was earlier inferred from this Laboratory[7] that the terminal α-galactosyl residue might be linked α-1,6 to N-acetylglucosamine and the increased inhibiting power of galactinol[27] over all other α-galactosides tested was considered in accord with this hypothesis. Molecular models were compatible with this interpretation.[3,27] Morgan, however, has recently reported[8] that galactosyl-α 1,3-galactose was a better inhibitor of B-anti-B hemagglutination than was melibiose. Galactinol itself cannot be a constituent of blood group B substance since inositol is absent; nor was the galactosyl-1,3 α-galactose obtained from blood group substance. Watkins[28] also mentioned that enzyme-synthesized[29] galactosyl 1,6-α-N-acetylglucosamine was inactive in hemagglutination inhibition tests, but details have not been published. The heterogeneity of antibodies in the various sera makes many sera not suitable for hemagglutination inhibition assay with small oligosaccharides.[30] A final determination of the nature of the second unit of the B specific grouping must await the availability of the different galactosyl-α-N-acetylglucosamines and the galactosyl-α-galactoses and the determination of their relative potencies in inhibiting both B-anti-B precipitation and the hemagglutination of B cells by anti-B or the isolation from B substance of the terminal disaccharide and establishment that it is more active than galactinol or other disaccharides.

Since a molecular weight[3] of 300,000 is assumed for blood group B substance and since the 5.2 mg. of reducing sugar would equal 6.45 mg. of galactose

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(26) E. A. Kabat, *J. Cellular Comp. Physiol., Suppl.*, **50**, 79 (1957).
(27) E. A. Kabat, Ciba-Foundation Symposium on the Chemistry and Biology of Mucopolysaccharides, London, April 24, 25, 1957, J. and A. Churchill, London, 1958, p. 42.
(28) W. M. Watkins, Ciba-Foundation Symposium on the Chemistry and Biology of Mucopolysaccharides, London, April 24, 25, 1957; J. and A. Churchill, London, 1958, p. 60.
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(30) E. A. Kabat, *J. Immunol.*, **84**, 82 (1960).

per 102 mg. of this substance, this would calculate to 36 μmole split per 0.3 μmole blood group substance or about 110 galactoses per molecule. There is no necessity for all of these to be B specific groupings and, in addition, if chains of α-linked galactoses occurred, these would be split sequentially and the estimate of terminal galactose would be reduced. The value thus provides a theoretical upper limit for the number of B groups per molecule. A relatively small number of B specific groupings, however, would adequately account for precipitation and hemagglutination inhibition.

The present study opens numerous avenues for further investigation. The cross reactivity and residual precipitating power for anti-B of the enzyme-treated B and BP1 substances should be studied by oligosaccharide inhibition techniques. Further degradation with other enzymes may yield additional information about the sequences involved in the various specificities.

[CONTRIBUTION FROM THE DEPARTMENT OF PHARMACOLOGY, THE HEBREW UNIVERSITY-HADASSAH MEDICAL SCHOOL JERUSALEM, ISRAEL]

The Enzymatic Oxidation of 6-Mercaptopurine to 6-Thiouric Acid[1]

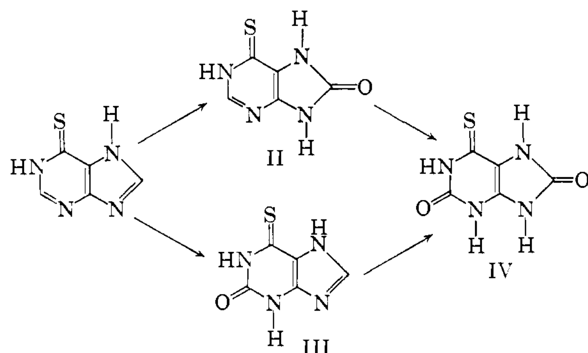
BY FELIX BERGMANN AND HANNA UNGAR

RECEIVED JANUARY 14, 1960

6-Mercaptopurine is attacked by xanthine oxidase first at carbon atom 8 and then at 2. The intermediate, 6-mercapto-8-hydroxypurine (II), cannot be isolated, as a result of its low concentration during the steady state of the reaction. However, II can be identified unequivocally by the use of isosbestic points. The pathway of oxidation of 6-mercaptopurine is different from that of hypoxanthine but corresponds to that of 4-hydroxypteridine.

From the urine of humans and mice, treated with 6-mercaptopurine (I), 6-thiouric acid (IV) has been isolated.[2] The metabolite is formed also *in vitro* under the catalytic influence of xanthine oxidase (XO).[3] The oxidation of I thus may appear to be in complete analogy with the conversion of hypoxanthine to uric acid. However, the pathway of the over-all reaction I → IV so far has not been elucidated.

In early experiments on this problem, we were unable to detect on paper chromatograms, developed at intermediate stages of the oxidation by XO, either of the two potential intermediates, *viz.*, 6-mercapto-8-hydroxypurine (II) or 6-thioxanthine (III).



The reason for our failure to isolate the intermediate became clear, when the enzymatic oxidation of synthetic II and III[4] was studied. In Fig. 1, the absorption spectra of both monohydroxy-6-thiopurines and of 6-thiouric acid are shown together. The conversion of II into IV can conveniently be measured at 305, 311 and 350 mμ. The results of a representative experiment are shown in Fig. 2, from which these various initial rates are derived: at 305 mμ, 101 μmole/h. ml.; at 311 mμ, 100 μmole/h. ml.; at 350 mμ, 115 μmole/h. ml. From these figures, the average relative rate, reported in Table I, was calculated.

TABLE I

PROPERTIES OF 6-MERCAPTOPURINE AND ITS DERIVATIVES

Compound	λ_{max} (mμ) at pH 8.0	Relative rate (%)[a]	R_F value in solvent[b] 1	2	3	Fluorescence
6-Mercaptopurine (I)	316	3.8	0.48	0.65	0.52	Yellowish
6-Mercapto-8-hydroxypurine (II)	311	23	.42	.58	.33	Pale blue-violet[c]
6-Thioxanthine (III)	341	46.5	.42	.53	.31	Yellow to white-blue
6-Thiouric acid (IV)	348		.08			Sky-blue

[a] All rates were measured with substrate concentrations of $6.5 \times 10^{-5}M$ and expressed as percentage of the rate of xanthine oxidation. [b] For composition of the solvents, see Experimental. [c] Compound II exhibits very weak fluorescence and thus cannot be visualized if less than 50γ is present.

The oxidation of 6-thioxanthine (III) was measured at 325 mμ (Fig. 3). The rate obtained, *viz.*, 200 μmoles/h. ml., is twice that of the oxidation of II.

As shown in Table I, the two potential intermediates are attacked by XO at rates about 6 and 12 times higher than the oxidation of 6-mercaptopurine. Therefore, already in the early part of the reaction a stage is reached where consumption of the intermediate keeps pace with its formation in the first step and thus prevents its accumulation.

However, the pathway of oxidation of I can be determined unequivocally by the proper use of isos-

(1) This work was supported in part by a grant from the U. S. Public Health Service, National Institutes of Health.

(2) G. B. Elion, S. Bieber and G. H. Hitchings, *Ann. N. Y. Acad. Sci.*, **60**, 297 (1954).

(3) (a) T. L. Loo, M. E. Michael, A. J. Garceau and J. C. Reid, THIS JOURNAL, **81**, 3039 (1959); (b) G. B. Elion, S. Mueller and G. H. Hitchings, *ibid.*, **81**, 3042 (1959).

(4) E. C. Moore and G. E. Le Page, *Cancer Research*, **18**, 1075 (1958), reported the enzymatic conversion of III into 6-thiouric acid but did not measure the rate.