ml. of 95% ethanol were refluxed for 15 minutes and distilled to dryness *in vacuo*. The residue was dissolved as far as possible in 15 ml. of water, and the resulting aqueous solution was filtered from the benzaldehyde phenylhydrazone formed, treated with activated charcoal, and evaporated to dryness *in vacuo*, yielding IV as a colorless oil.

Δ^1-Pyrroline-5-carboxylic Acid (V).—A solution of 273 mg. (1.0 millimole) IV in 5 ml. of 6 *N* hydrochloric acid was refluxed for 15 minutes. A portion of the resulting hydrolysate was diluted, neutralized with potassium carbonate, and immediately assayed with strain 55-25. The total activity found was very nearly equivalent to that of 1 millimole DL-proline. The yield of V was thus almost quantitative, if it is assumed that V is not more active than DL-proline. The hydrolysate was completely inactive for strain 55-1, showing absence of proline. The volatile hydrolysis products could be removed by distilling to dryness *in vacuo*.[32] Hydrolysates of IV purified *via* its phenylhydrazone were clear and colorless, while unpurified IV gave dark brown solutions; however, even such crude hydrolysates were adequate to demonstrate the growth response of the mutants. Crude IV gave 75 to 90% yields of V. In one experiment using crude IV the yield of V was studied as function of refluxing time; after 15 and 30 minutes, the yield was 90%, after 2 hours, 40%, and after 8 hours, 20%. Some proline was formed on prolonged heating, as shown by the response of strain 55-1. Hydrolysates of IV retained most of their biological activity for several weeks when stored at 3°; biological activity lost was partly recovered when the product was autoclaved at *p*H 7 in the minimal medium described.

Hydrogenation of Δ^1-Pyrroline-5-carboxylic Acid (V).—A hydrolysate, prepared by the method described from 300 mg. IV was evaporated to dryness *in vacuo*. The residue was immediately dissolved in 5 ml. of 80% aqueous acetic acid and shaken in hydrogen with 30 mg. Adams catalyst at 25° and atmospheric pressure until no more hydrogen was taken up. The reaction mixture was filtered from the catalyst and distilled to dryness. Assay of the resulting residue with strain 55-1 showed that a yield of 92 mg. of DL-proline (73% on the basis of IV) had been obtained. Assay with strain 55-25 gave the same yield, showing absence of V in the hydrogenated product. The formation of proline was confirmed by isolation as picrate.

DL-Proline Picrate.—The hydrogenated product described above, dissolved in water, was freed of chloride by successive treatment with silver carbonate and hydrogen sulfide. The resulting solution was treated with charcoal (Darco G-60) and evaporated to dryness. The residue was dissolved as far as possible in 10 ml. of 95% ethanol. The resulting solution was filtered from some undissolved material and distilled to dryness. The crystalline residue and 180 mg. of picric acid were dissolved in hot glacial acetic acid. The resulting product, after recrystallization from glacial acetic acid, weighed 140 mg. and melted at 136°.

Anal. Calcd. for $C_{11}H_{12}O_9N_4$: C, 38.38; H, 3.51; N, 16.28. Found: C, 38.35; H, 3.42; N, 16.01.

A mixed melting point with authentic DL-proline picrate[33] was undepressed and bioassay of the product and authentic picrate with strain 55-1 showed identical proline contents equal to the expected amount of about 33%.

Acknowledgments.—We are indebted to Roscoe C. Funk, Jr., Sloan-Kettering Institute for Cancer Research, for the microanalyses reported. The excellent technical assistance of Elizabeth S. Mingioli is gratefully acknowledged.

(32) The resulting residue or its aqueous solution were less stable than the original hydrolysate.

(33) D. Alexandroff, *Z. physiol. Chem.*, **46**, 17 (1905).

NEW YORK 21, N. Y. RECEIVED JULY 26, 1951

[CONTRIBUTION FROM THE EDWARD MALLINCKRODT DEPARTMENT OF PHARMACOLOGY OF WASHINGTON UNIVERSITY SCHOOL OF MEDICINE]

Enzymatic Synthesis of Desoxyxanthosine by the Action of Xanthosine Phosphorylase in Mammalian Tissue[1,2]

BY MORRIS FRIEDKIN

Desoxyxanthosine, the desoxyribosidic analog of xanthosine, has been isolated and characterized as the cyclohexylamine salt. The new purine desoxyriboside was formed enzymatically from xanthine and desoxyribose-1-phosphate by the action of xanthosine phosphorylase present in rat liver preparations. An analogous synthesis of xanthosine was carried out with xanthine and ribose-1-phosphate. The phosphorolysis of xanthosine and desoxyxanthosine occurs at a much slower rate than the phosphorolysis of desoxyguanosine.

Although xanthosine (xanthine riboside) has been known for some time,[3] the desoxyribosidic analog has not been described. During the course of experiments designed to increase the yield of desoxyribose-1-phosphate formed by the enzymatic phosphorolysis of desoxyguanosine[4] it was noticed that xanthine, one of the reaction products, unexpectedly disappeared with a concomitant loss of desoxyribose-1-phosphate. These observations lead to the isolation of the hitherto undescribed nucleoside: desoxyxanthosine (xanthine desoxyriboside) which was formed by an enzymatic reaction between xanthine and desoxyribose-1-phosphate as shown in reaction (1).

A completely analogous reaction between xanthine and ribose-1-phosphate with the formation of xanthosine was also shown to occur.

Indirect Enzymatic Formation of Desoxyxanthosine from Desoxyguanosine.—The enzyme solutions used in the present study were known from previous studies to catalyze reactions (2) and (3).[4] Desoxyguanosine + inorganic P $\rightleftarrows$ guanine + desoxyribose-1-phosphate (2). Guanine $\rightarrow$ xanthine (3)

Despite the reversibility of reaction (2) a good yield of desoxyribose-1-phosphate was predicted because of the probable irreversibility of reaction (3).[5] Thus for each mole of desoxyguanosine which undergoes enzymatic phosphorolysis, one mole of xanthine should also be formed owing to

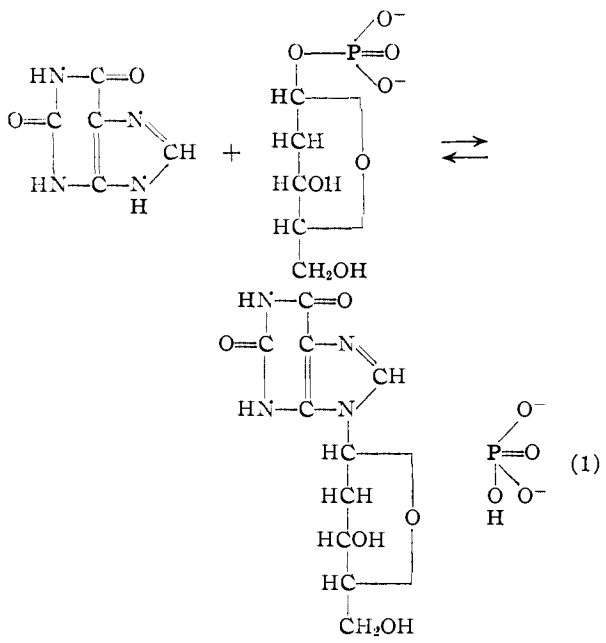
(1) Presented before the Division of Biological Chemistry at the 118th Meeting of the American Chemical Society, Chicago, Ill., September, 1950.

(2) This investigation was supported (in part) by a research grant from the National Institutes of Health, Public Health Service.

(3) P. A. Levene and W. A. Jacobs, *Ber.*, **43**, 3150 (1910); W. Jones, *J. Biol. Chem.*, **9**, 169 (1911); S. J. Thannhauser and B. Ottenstein, *Z. physiol. Chem.*, **114**, 2 (1921).

(4) M. Friedkin and H. M. Kalckar, *J. Biol. Chem.*, **184**, 437 (1950).

(5) This is an assumption which may not be true. The reversibility of guanine deaminase activity appears never to have been fully studied.



(1)

the action of guanase present in the rat liver preparation.

TABLE I

INCUBATION OF DESOXYGUANOSINE WITH RAT LIVER PREPARATION[a]

Time, minutes	Xanthine,[b] μmoles	Desoxyguanosine,[c] μmoles	Esterified P,[d] μmoles	Ratio of xanthine: esterified P
3	5.1	77	3	1.7
10	7.8	75	5	1.6
61	27	46	23	1.2
117	43	27	40	1.1
197	49	4.9	42	1.2
240	36	..	35	1.0
301	20	−0.9	24	0.8

[a] The incubation mixture consisted of 20 mg. of desoxyguanosine[6] (75 μmoles), 0.375 ml. of 0.2 *M* K_2HPO_4 (75 μmoles), 0.45 ml. of rat liver preparation (6 mg. of protein), and 7.5 ml. of 0.1 *M* tris-(hydroxymethyl)-aminomethane–HCl buffer, *p*H 7.4; temperature, 27°. At intervals as indicated in the table 0.2-ml. aliquots were removed. [b] Aliquot was heated at 100° for one minute, cooled, diluted to 50 ml. with 0.02 *M* phosphate buffer, *p*H 7.5, and filtered. To 3.0 ml. of the filtered sample was added 50 μl. of xanthine oxidase. The amount of free xanthine present was proportional to the increase of optical density at λ 293 mμ and was calculated on the basis, $\Delta E_{293} = +0.063$ for 1 μg. per ml., xanthine → uric acid (*p*H 7.4).[7,8] [c] To another 3.0 ml. of filtered sample obtained as indicated in footnote *b* were added 50 μl. of xanthine oxidase plus 50 μl. of diluted rat liver preparation (163 μg. of protein). The increase of optical density at λ 293 mμ in this case was equivalent to xanthine plus desoxyguanosine. After correcting for the amount of free xanthine present, the desoxyguanosine concentration was calculated on the basis, $\Delta E_{293} = +0.036$ for 1 μg. per ml., desoxyguanosine → uric acid (*p*H 7.4). [d] To an aliquot were added 50 μl. of 15 *M* NH_4OH and 50 μl. of 0.5 *M* $MgCl_2$–5.0 *M* NH_4Cl. After 15 hours at 3° the mixture was spun. The supernatant fluid was carefully removed with a micropipet and to the $MgNH_4PO_4$ precipitate was added 2.0 ml. of 3% perchloric acid. The mixture was chilled for one hour and then centrifuged for the removal of a slight amount of protein precipitate. One ml. was taken for the determination of inorganic phosphate according to the method of Gomori.[9]

(6) M. Friedkin, *J. Biol. Chem.*, **184**, 449 (1950).

(7) Optical density = log $(I_0/I)_\lambda = E\lambda$. All spectrophotometric measurements in this paper were made with 1-cm. cells.

(8) E. G. Krebs and E. R. Norris, *Arch. Biochem.*, **24**, 49 (1949).

(9) G. Gomori, *J. Lab. Clin. Med.*, **27**, 955 (1942).

As indicated in Table I when desoxyguanosine (75 μ moles) and an equimolar amount of inorganic phosphate were treated with the rat liver preparation 49 μ moles of xanthine were released and the concentration of esterified phosphate increased to 42 μ moles during the early period of the incubation. However during the last 104 minutes a considerable portion of the xanthine and of the esterified phosphate disappeared. After a total of 301 minutes of incubation enzymatic spectrophotometric analysis showed the presence of only 20 μ moles of xanthine. The amount of desoxyribose-1-phosphate had decreased to 24 μ moles and there was no detectable quantity of desoxyguanosine left. The fate of 55 μ moles of desoxyguanosine could not be accounted for.

The absorption spectrum of the incubation mixture indicated the presence of an unknown substance with maxima at λ 248 and λ 280 mμ in alkaline medium. After acid hydrolysis free xanthine was released and the maximum at λ 248 mμ disappeared. The absorption spectrum of the hydrolyzed mixture was characteristic of xanthine with a single maximum at λ 285 mμ in alkaline medium.[10]

These observations appeared to be consistent with the idea that xanthine, a supposedly inert end-product, was reacting with desoxyribose-1-phosphate to form desoxyxanthosine as shown in reaction (1). Desoxyxanthosine was subsequently isolated from the incubation mixture, first as a crude alcohol-insoluble barium salt and finally as the crystalline salt of cyclohexylamine. When analyzed for xanthine (after acid hydrolysis), desoxyribose and cyclohexylamine by methods described in the experimental part, the salt was found to contain these three constituents in equimolar amounts. As shown in Table II the absorption spectrum of cyclohexylamine desoxyxanthosine very closely resembles that of xanthosine. Both compounds exhibit similar spectral shifts with change of *p*H. Gulland and his collaborators assigned a 9-ribosidic linkage to xanthosine based on comparative spectrophotometric studies with the 7- and 9-methyl derivatives of xanthine.[11] It is similarly concluded from the spectrophotometric data that desoxyxanthosine is the 9-desoxyriboside of xanthine.

TABLE II

COMPARISON OF ABSORPTION SPECTRA OF CYCLOHEXYLAMINE DESOXYXANTHOSINE AND XANTHOSINE

Medium	Cyclohexylamine desoxyxanthosine Maximum, mμ	ε	Xanthosine[a] Maximum, mμ	ε
*p*H 10–12	248	10050	247	8600
	278	9090	278	7400
*p*H 7.5	248	10230	...	..
	278	8870		
*p*H 4-5	234	8340	238	7800
	263	9100	264	8400

[a] Values from paper of Gulland, *et al.*[11]

Acid Lability of Desoxyxanthosine.—Desoxyxanthosine is very rapidly split in dilute acid

(10) R. D. Hotchkiss, *J. Biol. Chem.*, **175**, 315 (1948).

(11) J. M. Gulland, E. R. Holiday and T. F. Macrae, *J. Chem. Soc.*, 1639 (1934).

even at room temperature whereas xanthosine under the same conditions is stable. The splitting can be followed by direct spectrophotometric measurement because of the sharp decrease of absorption which occurs at λ 235 mμ.

Direct Enzymatic Formation of Desoxyxanthosine and Xanthosine.—A more direct enzymatic synthesis of desoxyxanthosine was demonstrated by incubating xanthine with desoxyribose-1-phosphate in the presence of the rat liver preparation. Approximately 92% of the xanthine originally present was converted to a bound product presumably desoxyxanthosine. This product upon acid hydrolysis yielded free xanthine. During the incubation inorganic phosphate was released indicating the splitting of desoxyribose-1-phosphate. The ratio of inorganic phosphate released to the amount of xanthine bound was 0.88.

Analogous experiments were carried out with xanthine and ribose-1-phosphate. When 2.1 μ-moles of xanthine was incubated with 4 μ moles of ribose-1-phosphate and the rat liver preparation, 81% of the xanthine was converted to the riboside within 130 minutes.

Experimental

Preparation of Rat Liver Nucleoside Phosphorylase.—The previously described method of Kalckar[12] was modified as follows: Livers from 10 adult rats were perfused with ice-cold 0.9% NaCl and homogenized in a Waring blendor with 2 volumes of ice-cold 0.02 *M* phosphate buffer, *p*H 7.5. The homogenate was spun at 4000 r.p.m. The supernatant fluid was adjusted to 0.4 saturation with respect to ammonium sulfate by adding the calculated amount of saturated ammonium sulfate solution (25°). After spinning down the precipitate the supernatant was adjusted to 0.6 saturation by the further addition of ammonium sulfate. The precipitate was centrifuged, washed with a small volume of 0.6 saturated ammonium sulfate solution, redissolved in cold redistilled H_2O to a volume of 50 ml., and dialyzed for 15 hours at 3°. Denatured protein which precipitated during the dialysis was spun down and discarded. The fractionation with ammonium sulfate was repeated as above, the protein fraction, 0.4–0.6 was collected, redissolved, dialyzed for two days against redistilled H_2O and finally stored at −20°. Denatured protein appeared upon repeated freezings and thawings over a period of several months and whenever encountered was removed by centrifugation. Protein concentrations reported in this paper were determined spectrophotometrically according to the equation, protein (mg. per ml.) = $1.45\,E_{280} - 0.74\,E_{260}$.[12]

Isolation of the Cyclohexylamine Salt of Desoxyxanthosine.—Four hundred mg. of desoxyguanosine, 150 ml. of 0.1 *M* tris-(hydroxymethyl)-aminomethane–HCl buffer, *p*H 7.4, 7.5 ml. of 0.2 *M* K_2HPO_4 and 5.0 ml. of rat liver nucleoside phosphorylase (111 mg. of protein) were incubated at 30° for 3.5 hr. The enzymatic reactions were terminated by the addition of 15 ml. of 15 *M* NH_4OH and 30 ml. of 0.5 *M* $MgCl_2$–5.0 *M* NH_4Cl. After 15 hr. in a refrigerator the mixture was centrifuged for the removal of $MgNH_4PO_4$. To the clear supernatant was added 30 ml. of ammoniacal barium acetate (20 g. of $Ba(C_2H_3O_2)_2 \cdot H_2O$, 6 ml. of 15 *M* NH_4OH and 100 ml. of H_2O) and 950 ml. of 95% ethanol. After standing in a refrigerator for 15 hr. the mixture was centrifuged. The precipitate was washed several times with ethanol and finally with diethyl ether. After drying *in vacuo* at room temperature the crude barium salt weighed 620 mg. The barium salt was then extracted four times with 6.0-ml. portions of H_2O, and to the H_2O extract was added 360 ml. of dry *n*-butanol. Upon stirring a one-phase solvent system was formed owing to the solubility of water in butanol. The barium precipitate which formed was centrifuged, washed with diethyl ether and dried *in vacuo*. The yield of purified barium salt was 233 mg. To this salt taken up in 2.6 ml. of H_2O was added 157 mg. of cyclohexylamine sulfate in 0.67 ml. of H_2O. The $BaSO_4$ precipitate was centrifuged and washed once with 1.0 ml. of H_2O. The supernatant and wash were combined and mixed with 65 ml. of dry *n*-butanol. A slight amount of precipitate was spun down and discarded. To the clear wet butanol solution were added 85 ml. of diethyl ether. The mixture was seeded with a few crystals of cyclohexylamine desoxyribose-1-phosphate.[6] After 15 hr. at 3°, the precipitate was collected and after recrystallization from wet butanol–ether yielded 21 mg. of pure cyclohexylamine desoxyribose-1-phosphate.

The supernatant after removal of desoxyribose-1-phosphate was evaporated *in vacuo* in a bath at 40° to a volume of 30 ml. Diethyl ether, 210 ml., was added. The mixture on standing at 3° yielded 63 mg. of a deliquescent precipitate. The precipitate was dissolved in 0.6 ml. of H_2O and 9.0 ml. of *n*-butanol added with stirring. Upon the addition of 10 ml. of diethyl ether a slight amount of precipitate formed. This precipitate was spun down and discarded. Diethyl ether was added to the supernatant until a faint cloudiness appeared. The dense stubby crystals of cyclohexylamine desoxyxanthosine which formed on standing at 3° for three days were collected, washed two times with diethyl ether and dried *in vacuo* at 25°; weight, 15 mg. The salt sintered at 140–145° and decomposed at 145–151°. Another run essentially similar to the one just described yielded 17 mg. of cyclohexylamine desoxyxanthosine.

Analyses of Cyclohexylamine Desoxyxanthosine. A. Xanthine.—A solution containing 19.54 μg. of cyclohexylamine desoxyxanthosine per ml. of 0.06 *N* HCl was allowed to stand at room temperature for 40 min. The amount of xanthine present was calculated on the basis, λ_{max}^{acid} 265 mμ, ε 9098 (xanthine standard was twice recrystallized and dried *in vacuo* at 130°). Another sample of hydrolyzed cyclohexylamine desoxyxanthosine was neutralized and xanthine oxidase added as described in footnote (*b*) Table I. **B. Desoxyribose.**—The amount of desoxyribose present was determined by the Dische cysteine–H_2SO_4 method as modified by Stumpf.[13] Recrystallized desoxyguanosine was used as the standard. **C. Cyclohexylamine.**—The amount of cyclohexylamine present was determined by a colorimetric procedure developed in this Laboratory for measurement of amines and is based on the reaction between dinitrofluorobenzene and amino groups.[14] To 2.0 ml. of 3% $Na_2B_4O_7 \cdot 10\,H_2O$ (*p*H 9.15) containing 40 to 300 μmoles of cyclohexylamine per ml. were added 25 μl. of dinitrofluorobenzene in ethanol (382 μmoles per ml.). After 30 min. at 60° the tubes were cooled and acidified by the addition of 200. μl. of 12 *N* HCl. The insoluble dinitrophenyl derivative of cyclohexylamine was brought into solution by the addition of 2.0 ml. of glacial acetic acid. The optical density of λ 420 mμ was then determined.

	Anal. Calcd. for $C_{16}H_{25}O_6N_5$	Found
Xanthine ($C_5H_4O_2N_4$)	41.4	41.4 (based on absolute spectrum) 43 (based on enzymatic spectrophotometric analysis)
Desoxyribose ($C_5H_{10}O_4$)	36.5	40
Cyclohexylamine ($C_6H_{13}N$)	27.0	30

Hydrolysis of Desoxyxanthosine in Dilute Acid.—To 2.0 ml. of solution (145 mμ moles of desoxyxanthosine per ml.) in a Beckman cuvette was added 2.0 ml. of 0.12 *N* HCl at 22°. The decrease of optical density at λ 235 mμ was noted immediately. At intervals aliquots of the hydrolysis mixture were removed, neutralized, and analyzed for free xanthine (see footnote *b*, Table I). During the period of hydrolysis, two–eight minutes, the optical density change at λ 235 mμ was −0.183 which corresponded to the release of 30.4 mμ moles of xanthine per ml. of hydrolysis mixture. Upon complete hydrolysis of desoxyxanthosine the absorption spectrum of the hydrolyzate corresponded to xanthine. It was found that one mole of cyclohexylamine desoxyxanthosine (calculated for molecular weight, 367) released one mole of free xanthine upon acid hydrolysis.

(12) H. M. Kalckar, *J. Biol. Chem.*, **167**, 461 (1947).

(13) P. K. Stumpf, *ibid.*, **169**, 367 (1947).

(14) F. Sanger, *Biochem. J.*, **39**, 507 (1945).

Enzymatic Synthesis of Desoxyxanthosine with Xanthine and Desoxyribose - 1 - phosphate.—Cyclohexylamine desoxyribose-1-phosphate (2.9 μmoles)[6] and xanthine (1.72 μmoles) in 0.70 ml. of 0.1 *M* tris-(hydroxymethyl)-aminomethane–HCl buffer *p*H 7.4 were incubated with 78 μl. of rat liver preparation (1 mg. of protein) at 25°. After 106 minutes, 1.61 μmoles of xanthine were converted to a bound form from which xanthine could be completely recovered after acid hydrolysis in 1 *N* HCl (one minute at 100°). Xanthine was determined as described in footnote (*b*) Table I. During the same period of incubation, 1.42 μmoles of inorganic P was released (see footnote (*d*) Table I for determination of inorganic P). In a separate experiment involving the incubation of xanthine with desoxyribose-1-phosphate the absorption spectrum of an aliquot taken after two hours of enzymatic reaction exhibited the characteristic double maxima of desoxyxanthosine. Hydrolysis of an aliquot in 0.06 *N* HCl at room temperature resulted in a pronounced decrease of optical density at λ 235 mμ within a few minutes. After 10 minutes of hydrolysis the complete absorption spectrum was that of free xanthine.

Enzymatic Synthesis of Xanthosine with Ribose-1-phosphate and Xanthine.—The dipotassium salt of ribose-1-phosphate[15] (4 μmoles) was incubated with 2.1 μmoles of xanthine, 80 μl. of rat liver preparation (1.8 mg. of protein) and 733 μl. of 0.1 *M* tris-(hydroxymethyl)-aminomethane–HCl buffer, *p*H 7.4; temperature, 25°. Figures showing the disappearance of free xanthine are given (see footnote *b*, Table I for determination of xanthine):

Time, minutes	Xanthine, μmoles
2	1.7
30	0.61
106	0.45
130	0.40

The incubation was terminated at 140 minutes by heating at 100° for two minutes. The denatured protein was spun down and to 48.8 μl. of the clear supernatant fluid (R) was added 3.0 ml. of 0.3 *N* NaOH. The absorption spectrum showed maxima at λ 248 and λ 280 mμ characteristic of xanthosine. The spectrum of a hydrolyzed aliquot (to 48.8 μl. of (**R**) heated at 100° for five minutes with 100 μl. of 2 *N* HCl was added 3.0 ml. of 0.3 *N* NaOH) exhibited a single maximum at λ 285 mμ characteristic of free xanthine.

Discussion

Earlier studies with various purine nucleosides pointed to hypoxanthine and guanine ribosides (or desoxyribosides) as the only substrates that could be split phosphorolytically by nucleosidase prepared from mammalian tissues.[16] Recently Wang and Lampen have prepared cell-free extracts of *L. pentosus* which release reducing sugar from xanthosine, adenosine and cytidine by a reaction which cannot be definitely attributed to either a phosphorolytic or hydrolytic mechanism.[17]

In the present study it was found that the rat liver preparation known to have high desoxyguanosine phosphorylase activity unexpectedly had xanthosine phosphorylase activity as well, as evidenced by the synthesis of the xanthine nucleosides starting with free xanthine and desoxyribose-1-phosphate or ribose-1-phosphate. The apparent lack of xanthosine phosphorylase activity in mammalian preparations described by previous investigators[18] is most probably due to the use of too dilute enzyme solutions. As shown by the data in Table III, the desoxyxanthosine phosphorylase activity of the rat liver preparation was about 1/45th that of desoxyguanosine phosphorylase activity. This finding explains why desoxyxanthosine could be detected in the presence of desoxyguanosine despite the fact that the same enzyme preparation acted on both nucleosides. Because of the large difference in the rate of splitting it was possible by proper dilution of the enzyme to split desoxyguanosine without noticeably affecting the desoxyxanthosine. As a result enzymatic spectrophotometric analyses of mixtures of xanthine, desoxyguanosine, and desoxyxanthosine could be accomplished.

TABLE III

COMPARISON OF RATES OF ENZYMATIC SPLITTING OF XANTHINE NUCLEOSIDES AND DESOXYGUANOSINE BY RAT LIVER[a]

(1) Substrate	(2) Enzyme concn., mg. of protein per ml. total assay mixture	(3) Rate of splitting,[b] mμ moles of uric acid per ml. formed per 10 min.	(4) Specific activity of enzyme, (3)/(2)
Desoxyxanthosine	0.51	3.7	7.25
Xanthosine	.51	4.2	8.2
Desoxyguanosine	.012	3.9	325

[a] To 2.0 ml. of 0.075 *M* K phosphate buffer, *p*H 7.5 containing 125 mμ moles of nucleoside per ml. were added 48.8 μl. of xanthine oxidase (activity: 61 mμ moles of uric acid per ml. per 10 min. with xanthine as substrate) and 48.8 μl. of rat liver preparation; temp., 25°. The rate of increase of optical density at λ 293 mμ was then determined. [b] Calculated on the basis, $\Delta E_{293} = 9.7$ at *p*H 7.5 when 1 μmole per ml. of any of these nucleosides are converted to uric acid.

The direct formation of desoxyxanthosine isolated in the present study by a deamination of desoxyguanosine not involving reactions (2), (3) and (1) appears to be unlikely despite the possibility for such a pathway. Wakabayasi has prepared guanosine deaminase freed of nucleosidase activity from rabbit liver.[19] Desoxyguanosine presumably could be deaminated by the same enzyme. The data presented in Table I indicating that xanthine was first released and then bound during the incubation of desoxyguanosine with the rat liver preparation appear to preclude desoxyguanosine deamination as the main mechanism of desoxyxanthosine formation in the present study. Furthermore the direct synthesis of desoxyxanthosine from xanthine and desoxyribose-1-phosphate could be demonstrated.

The microbiological activity of the above preparation of desoxyxanthosine has been investigated and found to be similar to that of thymidine and other desoxyribosides in eliciting a growth response in the B_{12}-requiring bacteria, *L. leichmannii*.[20] This finding suggests that desoxyxanthosine may be of physiological importance in the biosynthesis of desoxyribose nucleic acid. It is possible however that desoxyxanthosine is acting as a source of desoxyribose-1-phosphate in the microbiological system and does not lie in the direct pathway of nucleic acid synthesis.

ST. LOUIS 10, MISSOURI RECEIVED APRIL 9, 1951

(15) Very generously provided by Dr. Graham Webster of the Department of Biochemistry, Washington University Medical School.

(16) F. Schlenk, *Advances in Enzymol.*, **9**, 455 (1949).

(17) T. P. Wang and J. O. Lampen, *Federation Proc.*, **9**, 399 (1950); *J. Biol. Chem.*, **192**, 339 (1951).

(18) M. Dixon and R. Lemberg, *Biochem. J.*, **28**, 2065 (1934); H. M. Kalckar, *J. Biol. Chem.*, **167**, 477 (1947).

(19) Y. Wakabayasi, *J. Biochem. (Japan)*, **28**, 185 (1938).

(20) B. S. Schweigert, unpublished experiments.