Purification and Properties of Trans-N-Deoxyribosylase*

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MacNutt (1) was the first to demonstrate the presence of an enzyme that catalyzes the transfer of the deoxyribosyl radical of deoxyribonucleosides to purines and pyrimidines in extracts of three bacterial species that require deoxyribonucleosides for growth. In addition, the presence of a deoxyribonucleoside hydrolase was noted in *Lactobacillus helveticus* extracts. The reaction products were identified by chromatographic methods and microbiological assays; Kalckar *et al.* (2) verified the transfer nature of the reaction with the aid of C^{14}-adenine. Hoffmann (3) and Lampen (4) have reported briefly on similar enzymes in *Escherichia coli*.

The present paper describes some of the properties of a partly purified enzyme from *L. helveticus* extracts which we call "trans-N-deoxyribosylase" (MacNutt (1) named the enzyme "trans-N-glycosidase"). Auxiliary enzymes have been used to detect and determine the products of the reaction.

METHODS

Materials—The sodium salt of thymus DNA[1] was prepared by the method of Hammarsten (5). The DNA was hydrolyzed with crystalline deoxyribonuclease (Worthington Biochemical Corp.) by MacNutt's (1) procedure and the deoxyribonucleosides were isolated by the method of Anderson *et al.* (6).[2] The products were characterized by absorption spectra (1, 6), enzymatic assay of the constituent purines (7–10) and cytosine (11), and by melting points (1, 6). The following constants were found (absorbancies were measured in pH 7.0, 0.1 M phosphate): deoxyguanosine, E_{280}/E_{260} 0.67, decomposition point near 240°; deoxyinosine, E_{280}/E_{260} 0.25, decomposition point near 220°; thymidine, E_{280}/E_{260} 0.73, m. p. 181 to 182°; deoxycytidine hydrochloride, E_{280}/E_{260} 0.94, m. p. with decomposition point 169–173°. Another preparation of the deoxyribonucleosides starting with sperm DNA (Nutritional Biochemicals Corp.) and partly purified pancreatic deoxyribonuclease was equally successful.

Cytosine, deoxyadenosine, and 6-mercaptopurine were obtained from the California Foundation for Biochemical Research. Guanine, adenine, xanthine, thymine, uracil, and inosine were obtained from Nutritional Biochemicals Corp. Hypoxanthine was purchased from Schwartz Laboratories, Inc.

8-Azaguanine (5-amino-7-hydroxy-1-*v*-triazolo(*d*)-pyrimidine) was synthesized by the method of Bennett (12). It was characterized by absorption spectrum (E_{280}/E_{260} 1.17, pH 7) and the action of guanase (9). 8-Azaxanthine (5,7-hydroxy-1-*v*-triazolo(*d*)-pyrimidine) was synthesized from 8-azaguanine by the method of Roblin *et al.* (13). The findings were E_{280}/E_{260} 0.60, pH 7.0.

Enzymes—Guanase was prepared from rat brains by the method of Kalckar (14). The preparation was free of xanthine oxidase. Milk xanthine oxidase was prepared by the method of Kalckar and Klenow (15). Adenase was prepared from *Torulopsis utilis* by the method of Roush (10). This preparation contained guanase but was free of xanthine oxidase. Cytosine deaminase free of the above enzymes was prepared from *Saccharomyces cerevisiae* by the method of Kream and Chargaff (11). Intestinal phosphatase was prepared from calf intestines by the method of Schmidt and Thannhauser (16) up to the step before reprecipitation with 0.8 saturated ammonium sulfate. This preparation was used as a source of adenosine deaminase. Cytidine deaminase was obtained from *E. coli* as reported by Wang *et al.* (17). All of the auxiliary enzymes were dialyzed until free of phosphate to avoid interference by the possible presence of nucleoside phosphorylase.

Trans-N-Deoxyribosylase and Deoxyribonucleoside Hydrolase Assays—To measure trans-N-deoxyribosylase activity 50 μg. of a deoxyribonucleoside and 50 μg. of a purine or pyrimidine were placed in a spectrophotometer cell and diluted to 3 ml. with a suitable buffer. A detecting enzyme which would use one of the products of the reaction as a substrate was added in large excess and the reaction was started by the addition of 0.1 ml. of a suitable enzyme preparation. Absorbancy readings were taken in the spectrophotometer at the appropriate wave length for 30 minutes. The initial reaction rate (ΔE/hour) was determined from a plot of the data. In every experiment, control reactions were carried out with each of the reactants alone. This method permits the detection of the formation of a new compound; a decrease in one of the substrates was detected by combining a heating step with the use of an auxiliary enzyme. Hydrolase activity was measured in the same manner with the exception that the purine or pyrimidine acceptor was omitted from the reaction solution. Protein was determined by the method of Lowry *et al.* (8) using crystalline bovine serum albumin as the standard. The unit of specific activity is μmoles of product formed per hour per mg. of protein.

Growth of L. helveticus (ATCC Nos. 8018 and 10,386) and Preparation of Extracts—The bacteria were grown at 42° in Dubois flasks for 2 days on the following medium: 15 gm. of peptonized milk (Difco), 6 gm. of yeast extract (Difco), 20 gm. of glucose, 0.5 gm. of soluble starch, 1 ml. of Tween 80, 200 ml. of

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[1] The abbreviations used are: DNA, deoxyribonucleic acid; Tris, tris(hydroxymethyl)aminomethane.

[2] The authors gratefully acknowledge gifts of a generous supply of resins from the Dow Chemical Co. and from Rohm and Haas Co.

TABLE I

Purification of trans-N-deoxyribosylase and deoxyinosine hydrolase

Transferase activities were measured with the aid of milk xanthine oxidase and with guanine and deoxyinosine as substrates, by the assay described under "Methods." Hydrolase activities were measured in the same manner with the omission of guanine.

Fraction	Transferase		Hydrolase	
	Specific activity*	Total units	Specific activity*	Total units
1. Initial extract	0.032	145	0.010	45
2. First $(NH_4)_2SO_4$ fractionation	0.10	102	0.016	16
3. Second $(NH_4)_2SO_4$ fractionation	0.22	77	0.037	13
4. Heated fraction	0.36	82	0.057	13

* μmoles of uric acid formed per hour per mg. of protein.

whey (fresh milk), 10 gm. of calcium carbonate, and 800 ml. of water. The cells were collected by centrifugation and washed once with pH 6.0, 0.05 M citrate. The yield was 12 to 14 gm. of packed cells/liter of medium. 70 gm. of bacteria were suspended in 150 ml. of pH 7.0, 0.1 M Tris[3] buffer and disrupted with 3 mm. glass beads in an ice-jacketed Waring Blendor cup. The blades of the blender were covered with Tygon tubing and a rheostat was used to reduce the blade speed to a point where the glass beads were not broken. The suspension was centrifuged at 6000 $\times$ *g*, the residue was reextracted in the same manner as before with additional buffer, and the combined supernatant fluids were stored in a freezer at $-15°$. The frozen extracts retained their activity for more than 1 year. An attempt to stimulate enzyme production by the addition of sperm DNA or deoxyribonucleosides to the growth medium of the bacteria was unsuccessful.

RESULTS

Distribution of Trans-N-Deoxyribosylase—Extracts of various microorganism and animal tissues were tested for activity using deoxyinosine with adenine and with guanine as substrates. These tests were carried out in pH 8.5 Tris at 37°. Activity was found only in *L. helveticus* (ATCC Nos. 8018 and 10,386) and *E. coli* extracts. Negative results were obtained with *Lactobacillus delbrueckii* (ATCC No. 9649), yeast species of 10 genera, two molds, six bovine organs, rat liver, and the hepatopancreas of the crayfish (19).

Purification of Trans-N-Deoxyribosylase—The extract from 75 gm. of *L. helveticus* was fractionated with ammonium sulfate. The fraction precipitating between 0.4 and 0.7 saturation contained most of the activity. It was dissolved in 50 ml. of distilled water, dialyzed for 24 hours against distilled water, and subjected to a second ammonium sulfate fractionation. The fraction collected between 0.5 and 0.6 saturation with ammonium sulfate had the highest specific activity. This precipitate was dissolved in distilled water and dialyzed as before; no loss in activity was noted upon dialysis. The dialyzed solution was heated to 55°, held at 55° for 10 minutes, cooled, and centrifuged. The supernatant fluid contained 57 per cent of the activity of the original extract and an 11-fold increase in specific activity had been effected. The results of this fractionation are given in Table I; deoxyinosine hydrolase activities are also included. The purified enzyme was stored in the freezer and retained its activity for at least a year. Ordinarily 0.1 ml. of a 1:10 dilution was used in activity tests.

[3] pH 6.0 phosphate and acetate buffers were also used successfully for the extraction.

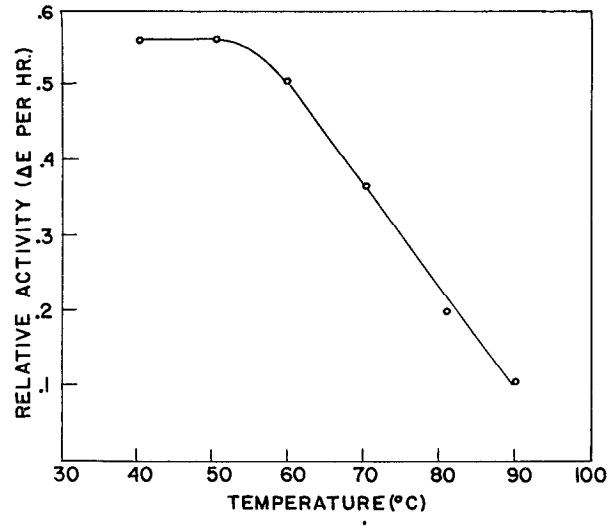

FIG. 1. Thermal inactivation of trans-N-deoxyribosylase. 0.2 ml. of purified enzyme was added to 1 ml. of pH 6.3 buffer, 0.02 M with respect to both Tris and acetate. The enzyme was held in a water bath at the indicated temperature for 10 min., cooled, and refrigerated until the residual activity was measured. 0.2 ml. of the heated enzyme was used to determine the residual activity with deoxyinosine and guanine as the substrates and with xanthine oxidase as the auxiliary enzyme.

Thermal Inactivation—Fig. 1 shows the results of heating the enzyme (Fraction 3) for 10 minutes at pH 6.3. The transferase

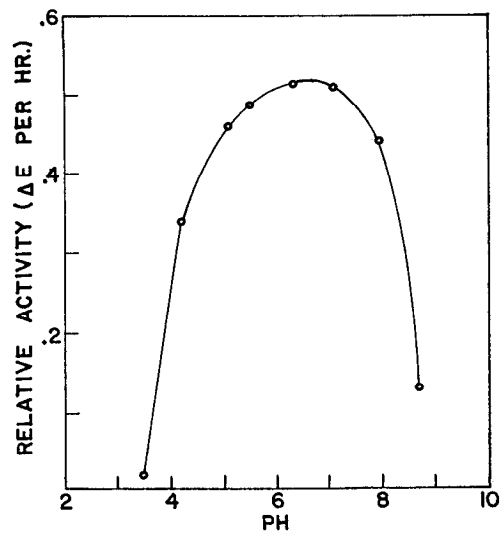

FIG. 2. pH stability. 0.2 ml. portions of purified enzyme were added to 1 ml. portions of the appropriate buffers (pH 3 citrate and pH 4 to 9 Tris-acetate buffers), the diluted enzyme was held at 61° for 30 minutes, cooled, and the residual activity determined with 0.2 ml. of the diluted enzyme. The pH of the diluted enzyme was measured with the glass electrode. The residual activity was determined with deoxyinosine and guanine as the substrates and with xanthine oxidase as the auxiliary enzyme.

is stable below 55° under the conditions of the experiment. About one-fourth of the activity remains after heating to 90°.

pH Stability—This is shown in Fig. 2. The pH of maximum stability of the enzyme is near 6.5.

pH Optimum—The pH activity curve of the enzyme is given in Fig. 3; the pH of optimum activity is near 5.8.

Substrate Specificity—Table II lists transferase activity rates in Tris and acetate buffers with deoxyinosine as the deoxyribosyl donor and various purines and pyrimidines as deoxyribosyl acceptors. Control reactions with the pyrimidines and purines with the exception of adenine gave rates that were near zero; the slight reaction observed with adenine is probably due to the direct action of xanthine oxidase on the adenine. In acetate buffer, higher rates are observed with the purines than with pyrimidines. 8-Azaguanine, a xanthine oxidase inhibitor (20), but not 8-azaxanthine, acts as a substrate. In Tris buffer, the pyrimidines and 8-azaguanine do not serve as substrates. Table III lists activity rates in phosphate and Tris buffers; again the purine gives a higher rate than the pyrimidine. There is no effect of phosphate on the cleavage of deoxyinosine; in general, hydrolase activity rates have been found to be about the same whether measured in phosphate, acetate or Tris buffers, indicating the absence of nucleoside phosphorylase. Table IV, a summary of substrate specificity studies with five deoxyribosyl compounds employing six detecting enzymes, shows a wide variety of transfers in acetate buffer but transfer only to or from purines in Tris buffer. In a later experiment carried out in phosphate buffer, 6-mercaptopurine was found to be a substrate when used in conjunction with deoxyinosine; the rate with both present was 0.154 ΔE/hour and with the latter alone the rate was 0.052 ΔE/hour. Attempts to exchange the guanine of thymus DNA with adenine using guanase as the detecting enzyme or to exchange the DNA adenine with guanine using adenase as the detecting enzyme were unsuccessful.

The Inhibition of Deoxyribosyl Transfer to and from Pyrimidines in Tris Buffer—This reaction is completely inhibited in Tris buffer (Tables II and III). Fig. 4 shows the increasing inhibition of the enzyme-catalyzed transfer of the deoxyribosyl group from hypoxanthine to cytosine in phosphate buffer by the addition of increasing concentrations of Tris buffer. Tris was found to have no effect on the xanthine oxidase reaction with hypoxanthine as the substrate and did not inhibit the hydrolase reaction using deoxyinosine as the substrate. That the decrease in rate in Tris buffer was not caused by denaturation was shown by dilution of the concentrated enzyme with Tris buffer, allowing the diluted enzyme to stand 1 hour, and using the diluted enzyme to catalyze the reaction in phosphate buffer; the rate was the same as with enzyme diluted with water or phosphate. Table V shows that the inhibition of transfer to and from pyrimidines in Tris buffer can be reversed by phosphate. No reversal was found with acetate, chloride, or sulfate.

The Inhibition of Deoxyribosyl Transfer to and from Purines in Tris Buffer—A partial inhibition of transfer to and from purines in Tris buffer has been noted throughout this work. Table VI shows the effect of Tris on the transfer of the deoxyribosyl radical from hypoxanthine to guanine. Complete inhibition of this reaction has not been observed; 0.017 M Tris reduces the rate of transfer to purines to about 70 per cent of the rate in phosphate alone. Increasing concentrations of Tris do not reduce the rate further.

Stoichiometry of the Trans-N-Deoxyribosylase Reaction—For

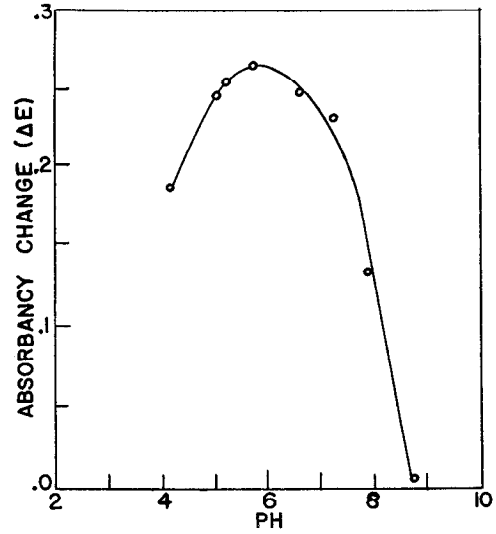

FIG. 3. pH of optimum activity. 0.1 ml. of the purified enzyme was added to 2 ml. of the appropriate buffer containing 50 μg. of guanine and 50 μg. of deoxyinosine. 0.02 M Tris-acetate buffers, pH 4 to 9 were used. (Similar solutions were prepared for pH measurement.) The reaction solution was incubated 10 minutes at 37°, 2.0 ml. of pH 8.1, 0.3 M Tris buffer were added, the tube heated 5 minutes in a boiling water bath, and cooled. 3 ml. of the resulting solution were used for the determination of hypoxanthine with xanthine oxidase. The pH of these solutions varied from 7.9 to 8.1.

TABLE II

Transfer of the deoxyribosyl group of deoxyinosine

The appearance of hypoxanthine was measured with the aid of milk xanthine oxidase by the assay described under "Methods."

Substrates	ΔE_{293} per hr.	
	pH 6.2 acetate	pH 7.0 Tris
Deoxyinosine +		
Adenine	1.494	0.534
Guanine	1.383	0.256
Uric acid		0.008
8-Azaguanine	0.173	0.004
8-Azaxanthine	0.122	0.007
Cytosine	0.546	0.002
Thymine	0.236	0.010
Uracil	0.220	0.000
Deoxyinosine (alone)	0.113	0.010
Adenine (alone)	0.024	0.052

TABLE III

Transfer of the deoxyribosyl group of deoxyinosine in phosphate and Tris buffers

The appearance of hypoxanthine was measured with the aid of milk xanthine oxidase by the assay described under "Methods."

Substrates	ΔE_{293} per hr.	
	pH 6.1, 0.1 M phosphate	pH 6.9, 0.1 M Tris
Deoxyinosine + guanine	2.590	1.920
Deoxyinosine + cytosine	0.974	0.002
Deoxyinosine (alone)	0.197	0.282

TABLE IV

Summary of the results of substrate specificity studies

Experiment no.	Substrates tested		Enzyme used in assay	Substrates giving positive results	
	Deoxyribosyl compounds	Purines and pyrimidines		pH 6.2 acetate	pH 7.0 Tris
1	dI*	Adenine, guanine, uric acid, azaguanine, azaxanthine, cytosine, thymine, and uracil	Xanthine oxidase	All but azaxanthine (uric acid not tested)	Adenine and guanine
2	dG	Adenine, hypoxanthine, xanthine, azaxanthine, cytosine, thymine, and uracil	Guanase	All but azaxanthine	Adenine, hypoxanthine, xanthine
3	dA	Hypoxanthine, xanthine, azaxanthine, cytosine, thymine, uracil	Adenase	All but axaxanthine	Hypoxanthine, xanthine
4	dC	Adenine, guanine, hypoxanthine, xanthine, azaxanthine, thymine, uracil	Cytosine deaminase	All but azaxanthine	
5	dG, dI, dC, T	Adenine	Adenosine deaminase	All	dI, dG
6	dA, dG, dI, T	Cytosine	Cytidine deaminase	All	

* The abbreviations used in Table IV and Table VII are: dA, deoxyadenosine; dC, deoxycytidine; dG, deoxyguanosine; and dI, deoxyinosine; T, thymidine.

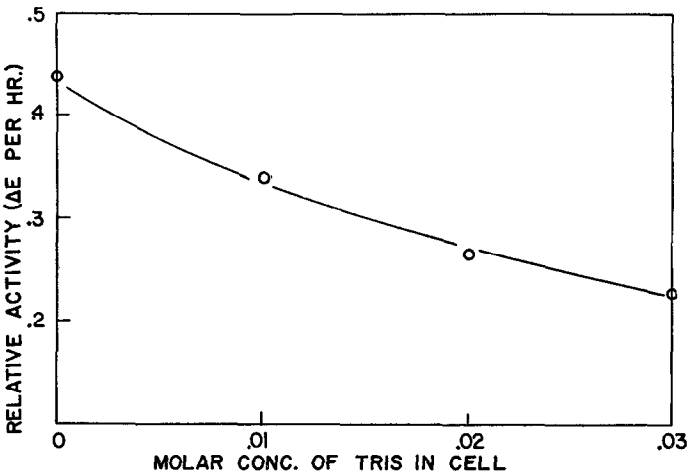

FIG. 4. The inhibition of transfer by Tris in the presence of phosphate buffer. Activities were measured with deoxyinosine and cytosine as the substrates and with xanthine oxidase as the auxiliary enzyme. Phosphate concentration was 0.33 M in each reaction.

TABLE V

The effect of phosphate on Tris inhibition of deoxyribosyl transfer from hypoxanthine to cytosine

Activities were measured with the aid of milk xanthine oxidase and with cytosine and deoxyinosine as substrates at pH 7.0 by the assay described under "Methods."

Experiment no.	Tris concentration	Phosphate concentration	ΔE_{293}/hr.
	molar	*molar*	
1	0.017	0.000	0.137
2	0.017	0.033	0.302
3	0.017	0.050	0.344
4	0.000	0.033	0.405

the reaction deoxycytidine + adenine = cytosine + deoxyadenosine, it was possible to determine each of the reactants and products with the use of the enzymes cytidine deaminase, adenase, cytosine deaminase, and adenosine deaminase, respectively. The results of such an experiment are given in Fig. 5 and it is evident that deoxycytidine and adenine decrease in concentration at about the same rate as cytosine and deoxyadenosine appear. Table VII gives data for this reaction and for several other similar reactions. An estimation of equilibrium constants is included; in these calculations it was assumed that equilibrium is attained and that hydrolase action is negligible.

DISCUSSION

The above results confirm and extend the work of MacNut (1) who used paper chromatography and microbiological assay to detect trans-N-deoxyribosylase activity in bacterial extracts. The use of auxiliary enzymes to detect and determine products of this reaction has proved advantageous because of the rapidity of the spectrophotometric assay and because specific compounds can be determined readily.

The search for a better source of the transferase revealed a limited distribution of the enzyme; it was found only in species of bacteria where it had been detected previously (1, 3, 4). This leads to the conclusion that it has a highly specific role in nucleic acid metabolism in those organisms where it occurs and is not involved in a general metabolic pathway. Perhaps the function is similar to that of nucleoside phosphorylase in other species. MacNutt (1) carried out his reactions mainly in pH 8.5 alanylglycine buffer and, accordingly, our species distribution assays were carried out at the same pH in Tris buffer. Later, the pH optimum of the enzyme was found to be near pH 5.8 and therefore there is a slight possibility that the assays might have failed to detect the enzyme in extracts of some species because the reactions were run 2.7 pH units above this optimum. Inability to detect the transferase in *L. delbrueckii* might have resulted because of this reason, because of strain

TABLE VI

The effect of Tris on deoxyribosyl transfer from hypoxanthine to guanine

Activities were measured with the aid of milk xanthine oxidase using guanine and deoxyinosine as substrates at pH 7.0 by the assay described under "Methods."

Experiment no.	Phosphate concentration	Tris concentration	ΔE_{293}/hr.
	molar	*molar*	
1	0.017	0.000	1.018
2	0.017	0.017	0.642
3	0.017	0.033	0.654
4	0.017	0.067	0.666

differences, because of a Tris inhibition, or because the medium for *L. delbrueckii* contained natural nutrients in contrast to MacNutt's (1) medium where thymidine was employed.

The purification procedure resulted in an 11-fold increase in the trans-N-deoxyribosylase specific activity (per unit of protein) which was accompanied by a similar increase in deoxyinosine hydrolase specific activity. The enzyme preparations also catalyze the hydrolysis of pyrimidine and other purine deoxyribosyl compounds. These results might signify that a transfer and a hydrolase activity reside in the same enzyme. The transferase specific activity rates are about 6 times those of the hydrolase in all fractions. Hoffman (3) found that the arsenolysis of thymidine by *E. coli* preparations was inhibited by the presence of uracil because of the transfer reaction. These results are consistent with a preferential transfer of the deoxyribosyl group to the nitrogen of a purine or a pyrimidine rather than to the oxygen of water. Also, stoichiometry (Table VI) indicates

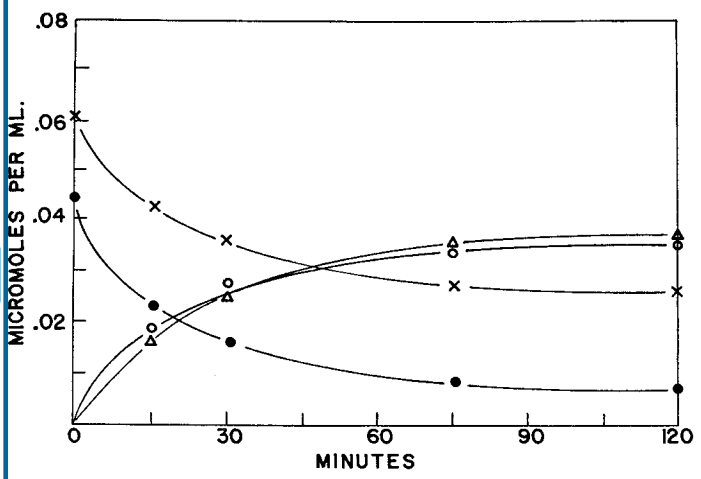

FIG. 5. Stoichiometry of the reaction of deoxycytidine and adenine. At zero time, 0.2 ml. of a 1:10 dilution of the purified enzyme was added to 40 ml. of a solution containing 1 mg. of adenine and 0.95 mg. of deoxycytidine in pH 6.0, 0.1 M acetate buffer. At various times 8 ml. samples were removed, placed in a boiling water bath for 5 minutes, cooled, and refrigerated until analyzed. To determine each of the compounds present, 1 ml. of the solution was diluted to 3 ml. with pH 6.0 acetate buffer and the total absorbancy change measured at the appropriate wave length after addition of an auxiliary enzyme (adenase, adenosine deaminase, cytosine deaminase, or cytidine deaminase). ×—×, adenine; ·—·, deoxycytidine; ○—○, deoxyadenosine; △—△, cytosine.

TABLE VII

*Stoichiometry of trans-N-deoxyribosylase reactions and estimated equilibrium constants**

Reaction†	Time	Decrease in reactant	Product formed	K
	min.	*μmole*	*μmole*	
1. dI‡ + guanine ⇌ dG + hypoxanthine	193	0.039	0.040	3.2
2. dG + hypoxanthine ⇌ dI + guanine	60	0.0093	0.0094	0.35
3. *dC* + adenine ⇌ *dA* + cytosine	120	0.036, 0.035	0.034, 0.037	5.6
4. dI + azaguanine ⇌ azaguanine deoxynucleoside + hypoxanthine	330	0.018	0.018	1.9
5. T + cytosine ⇌ *dC* + thymine	120	0.022	0.020	0.18

* See the legend of Fig. 5 for the method used in obtaining this data.

† Italicized compounds were determined.

‡ See Table IV for abbreviations.

a complete lack of hydrolase activity when a purine or a pyrimidine acceptor is present. Ott and Werkman (21) in a study of the nucleoside phosphorylase-catalyzed transfer of ribose from inosine to adenine note that "hydrolysis of ribose-1-phosphate does not occur to any appreciable extent when both adenine and inosine are present," *i.e.* transfer suppresses hydrolysis. No evidence has been found for a nucleoside phosphorylase in *L. helveticus*; however, it is possible that the active center of the transferase contains phosphate and that the mechansim of transfer is similar to transfer reactions mediated by nucleoside phosphorylase (21, 22).

The complete inhibition of transfer of the deoxyribosyl radical to and from pyrimidines by Tris and the partial inhibition of transfer from purines to purines is indicative of the presence of at least two enzymes in the preparations, one (not inhibited by Tris) catalyzing purine-purine transfers and the other (inhibited by Tris) catalyzing pyrimidine-pyrimidine, pyrimidine-purine, and possibly purine-purine transfers. Hoffmann (3) has presented evidence for two transferring enzymes in *E. coli*, one involving purine and one involving pyrimidine transfers. Further purification of the transferase will be necessary in order to decide if more than one enzyme is responsible for the various hydrolase and transfer activities.

The finding that 8-azaguanine and 6-mercaptopurine serve as substrates for trans-N-deoxyribosylase is of interest in that other enzyme-catalyzed reactions have been found for which these purine analogues serve as substrates.

The estimated equilibrium constants do not differ greatly from unity, as might be expected for reactions of this nature. ΔF^0 for the transfer reaction is small. The equilibrium involving deoxyinosine and guanine was approached from both directions (Table VI, Reactions 1 and 2) and the two equilibrium constants (3.2, 1/0.35 = 2.9) do not differ greatly, hence the assumptions involved in these calculations appear to be valid.

SUMMARY

Trans-N-deoxyribosylase appears to be of limited distribution in nature. The specific activity of this enzyme in *Lactobacillus*

helveticus extracts was increased 11-fold by ammonium sulfate fractionations and a heating step. It is quite stable to heat, with optimum stability near pH 6.5 and optimum activity near pH 5.8.

The transfer of the deoxyribosyl group of both purine and pyrimidine deoxyribonucleosides to guanine, adenine, hypoxanthine, 8-azaguanine, 6-mercaptopurine, thymine, uracil, and cytosine by this enzyme is demonstrated with the aid of auxiliary enzymes. Tris(hydroxymethyl)aminomethane buffer completely inhibits those transfers involving pyrimidines and partly inhibits transfers where only purines are involved as substrates. Phosphate partly reverses the inhibition of pyrimidine transfer reactions. The stoichiometry of these reactions has been studied and equilibrium constants estimated.

Cytosine deaminase, cytidine deaminase, adenase, deoxyadenosine deaminase, guanase, deoxyguanosine deaminase, xanthine oxidase, and nucleoside phosphorylase were not detected in *Lactobacillus helveticus* extracts.

REFERENCES

1. MacNutt, W. S., *Biochem. J.*, **50,** 384 (1952).
2. Kalckar, H. M., MacNutt, W. S., and Hoff-Jørgensen, E., *Biochem. J.*, **50,** 397 (1952).
3. Hoffmann, C. E., *Federation Proc.*, **11,** 231 (1952).
4. Lampen, J. O., In McElroy, W. D., and Glass, H. B., *Phosphorus metabolism, Vol. II*, Johns Hopkins Press, Baltimore, 1952, p. 369.
5. Hammarsten, E., *Biochem. Z.*, **144,** 383 (1924).
6. Anderson, W., Dekker, C. A., and Todd, A. R., *J. Chem. Soc.*, 2721 (1952).
7. Kalckar, H. M., *J. Biol. Chem.*, **167,** 429 (1947).
8. Kalckar, H. M., *J. Biol. Chem.*, **167,** 445 (1947).
9. Roush, A., and Norris, E. R., *Arch. Biochem.*, **29,** 124 (1950).
10. Roush, A. H., *Arch. Biochem. Biophys.*, **50,** 510 (1954).
11. Kream, J., and Chargaff, E., *J. Am. Chem. Soc.*, **74,** 5157 (1952).
12. Bennett, E. L., *J. Am. Chem. Soc.*, **74,** 2420 (1952).
13. Roblin, R. O., Lampen, J. O., Jr., English, J. P., Cole, Q. P., and Vaughan, J. R., Jr., *J. Am. Chem. Soc.*, **67,** 290 (1945).
14. Kalckar, H. M., *J. Biol. Chem.*, **167,** 461 (1947).
15. Kalckar, H. M., and Klenow, H., *J. Biol. Chem.*, **172,** 349 (1948).
16. Schmidt, G., and Thannhauser, S. J., *J. Biol. Chem.*, **149.** 369 (1943).
17. Wang, T. P., Sable, H. Z., and Lampen, J. O., *J. Biol. Chem.*, **184,** 17 (1950).
18. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. Biol. Chem.*, **193,** 265 (1951).
19. Roush, A. H., and Betz, R. F., *Biochim. et Biophys. Acta*, **19,** 579 (1956).
20. Lorz, D. C., and Hitchings, G. H., *Federation Proc.*, **9,** 197 (1950).
21. Ott, J. L., and Werkman, C. H., *Arch. Biochem. Biophys.*, **69,** 264 (1957).
22. Ott, J. L., and Werkman, C. H., *Biochem. J.*, **65,** 609 (1957).