ENZYMIC DEGRADATION OF DEOXYRIBOSE DERIVATIVES FOR DETERMINATION OF ISOTOPE DISTRIBUTION

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SUMMARY

A method for the degradation of deoxyribose applicable to the study of deoxyribose and DNA metabolism by tracer techniques is presented. The method utilizes a combination of *E. coli* extract and muscle extract to degrade DR-5-P to acetaldehyde and lactate which are degraded further to give each carbon as a single fraction. There appears to be no contamination with endogenous products and very little cross-over between different carbons of the deoxyribose. Comparison of data from enzymic and partial chemical degradation of deoxyribose confirms the validity of this fermentation procedure for determination of the distribution of tracer in deoxyribose.

INTRODUCTION

In studies on the biosynthesis of deoxyribose from ^{14}C-labeled precursors[1] it was necessary to have a reliable method for degrading deoxyribose to determine the distribution of isotope. The fermentation of deoxyribose by cell-free extracts seemed suitable for this purpose. HOFFMAN AND LAMPEN[2] found that *E. coli* fermented deoxyribose nucleosides to acetate, formate and ethanol and they postulated the intermediate formation of C_2 and C_3 fragments. RACKER[3] isolated an aldolase from extracts of *E. coli* which catalyzes the reversible conversion of deoxyribose-5-phosphate to acetaldehyde and triose phosphate. BERNSTEIN[4] has reported on the fermentation of isotopic deoxyribose nucleosides by whole cell preparations of *E. coli*.

In the present work, a cell-free extract of *E. coli* was used to degrade deoxyribose to acetaldehyde and triose phosphate. The acetaldehyde was oxidized chemically to acetic acid, and a rabbit muscle extract was used to convert triose phosphate to lactate. The acetate and lactate so obtained were then degraded chemically (see Fig. 1). In addition, two of the samples of the isotopically labeled deoxyribose (having different tracer patterns) were degraded by a chemical method described below.

The following abbreviations will be used: DPN, diphosphopyridine nucleotide; ATP, adenosine triphosphate; and DNA, deoxyribonucleic acid; DR-5-P, deoxyribose-5-phosphate.

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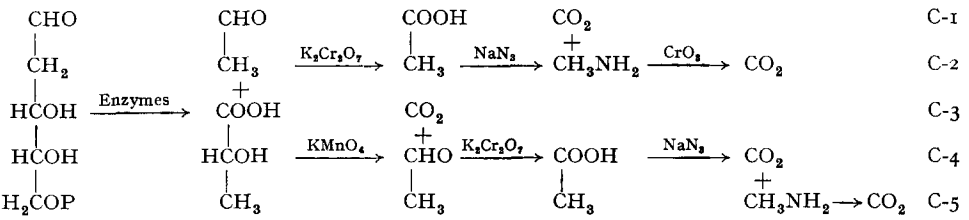

Fig. 1. Summary of the enzymic degradation procedure.

EXPERIMENTAL

Materials

Deoxyadenosine, thymidine, deoxyadenylic acid, deoxyguanylic acid and deoxyribose were purchased from the California Foundation for Biochemical Research. Barium fructose diphosphate from Schwarz Laboratories was acidified with H_2SO_4 and filtered to remove the $BaSO_4$ precipitate and the filtrate neutralized with NaOH. [2-^{14}C]Pyruvate was prepared by acid hydrolysis of [2-^{14}C]pyruvamide obtained from Volk Radiochemical Co. Sodium pyruvate was purchased from Nutritional Biochemicals, Inc. DPN and ATP were purchased from Pabst Laboratories and *Crotalus adamanteus* venom from Ross Allen's Reptile Institute. Dowex 50 (H^+ form) 50–100 mesh was washed with 0.1 *N* HCl and water prior to use. Duolite A-4 was obtained from Chemical Process Co. Celite 535 (Johns Manville) was washed with ether, chloroform, ethanol and water.

Deoxyribose-5-phosphate was prepared by acid hydrolysis of purine deoxynucleotides[5]. Deoxyadenylic or deoxyguanylic acid was heated at 100° C for 20 min in 0.1–0.15 *N* sulfuric acid and the solution was cooled and neutralized to pH 3.0 with saturated barium hydroxide and to pH 7.0 with potassium hydroxide. The barium sulfate was removed by filtration and the filtrate cleared of purine bases and cations by passage over a Dowex 50 (H^+) column. The eluate showed no absorption at 260 mμ.

Samples of [^{14}C]deoxyribose were obtained from the DNA of *E. coli*, R-2 or *E. coli* bacteriophage T_{2r^+h} grown on media containing a single ^{14}C-labeled carbon source[1]. The purine deoxynucleotides plus non-isotopic deoxyadenylic or deoxyguanylic acid added as carrier were hydrolyzed by heating in 0.1 *N* H_2SO_4 solution at 100° for 20 min. The solution was chilled and neutralized to pH 3.0 with saturated $Ba(OH)_2$ and to pH 7.0 with KOH and the precipitate of $BaSO_4$ filtered off. The filtrate was passed over a Dowex 50 (H^+ form) column. Non-isotopic adenine or guanine was added as an isotopic "wash" and the solution was again passed over Dowex 50 (H^+ form) columns until the solution showed no absorption at 260 mμ.

E. coli (9723 of the American Type Culture Collection) was used in this study as a source of deoxyribose aldolase. The cells were grown aerobically and a cell-free extract prepared as described by HOFFMAN AND LAMPEN[2]. Cells were grown in 50-l batches using 10 l of medium per 15-l bottle. The media were inoculated with 1/20 vol. of 4–6 h aerobic cultures. The bottles were incubated at 37° for 20 h with vigorous aeration. The cells (6–8 g, wet weight/l) were collected in a refrigerated Sharples centrifuge, washed once with 0.05 *M* $NaHCO_3$, twice with distilled water, and stored at —20°. For the preparation of extracts the cells were thawed and suspended in 0.9% NaCl at a concentration of 200 mg (wet weight) per ml. Cell suspensions were disrupted by sonic oscillation for 15 min at 4° in a water-cooled 50 W, 9 kc sonic

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oscillator (Raytheon Model R-22-3, 50 W, 9 kc). The crude extracts were partially purified exactly as described by HOFFMAN AND LAMPEN[2] and then frozen. This frozen extract retained activity at least 6 months but lost activity on repeated freezing and thawing. The activity of the extract was determined by measuring the rate of disappearance of deoxyribose in a reaction mixture containing 4.5 μmoles thymidine, 25 μmoles $NaHCO_3$, 7.5 μmoles phosphate buffer, pH 7.0, 0.25 ml of cell-free extract (equivalent to 50 mg cells wet weight) in a total volume of 2.5 ml. The tubes were incubated at 37° for 0, 20 and 40 min. The reaction was stopped with 1.0 ml of 10% $HClO_4$ and aliquots of the protein-free filtrates used for deoxyribose determination.

Rabbit muscle extract was prepared by a modification of the method of TAYLOR, GREEN AND CORI[6]. Two rabbits were anesthetized with nembutal and killed by exsanguination. Skeletal muscle was removed quickly, ground, and extracted in the cold, twice with 1 vol. of 0.04 *N* KOH and finally with ½ vol. of water. Saturated ammonium sulfate solution was added to the extract to give a 0.35 saturated solution. The precipitate was filtered and discarded. Saturated ammonium sulfate solution and the solid salt were added to the filtrate to give a concentration of 0.85 saturation. The precipitate was collected in a filter press*, and the moist cake stored in a screw-capped bottle at 3°. This preparation has not lost activity in nearly 2 years. For use, 5 g of the cake were dissolved in 60 ml of water and dialyzed overnight. To the dialysate enough solid KCl was added to make the solution 0.1 *M*, and solid $NaHCO_3$ added to adjust the pH to 7.0. This solution may be frozen once and retains activity for at least 3 months, but loses activity on repeated freezing and thawing. The extract was assayed as follows: 125-ml Warburg vessels containing 1.0 mmole of fructose diphosphate, 10 μmoles of DPN, 30 μmoles ATP, 20 μmoles $MgCl_2$, 200 μmoles K_2HAsO_4, 4.0 mmoles $NaHCO_3$, 5 ml of rabbit muscle extract (in a side-arm), in a final volume of 40 ml were gassed for 15 min with 95% N_2–5% CO_2 mixture, tipped, and incubated at 37° with shaking. CO_2 evolution was measured manometrically and the reaction was considered complete when CO_2 production ceased. In a typical fermentation, in 40 min, 39,000 μl or 1.7 mmoles of CO_2 were formed from 1 mmole of fructose diphosphate. Results were checked by lactic acid determinations in some assays.

Analytical methods

Deoxyribose was determined by the cysteine-sulfuric acid method of STUMPF[7,8]. Acetaldehyde was estimated either by an iodometric method[9] or by dichromate oxidation to acetic acid. The fermentation acids were separated and purified on columns of celite as described by BUEDING AND YALE[10]. Lactic acid was determined by the method of BARKER AND SUMMERSON[11].

Procedure for enzymic degradation of deoxyribose

Good yields of acetaldehyde and lactic acid were obtained when DR-5-P was used as the substrate and both bacterial and muscle extracts were added at the same time. The fermentation was carried out in a reaction train consisting of a 1000-ml suction flask with the side arm connected by rubber tubing to a trap and then to the inlet tube of a bead tower (Corning Glass Works drawing XA 3123) containing 25 ml of 2% $NaHSO_3$. The reaction vessel, containing 3.0 mmoles of DR-5-P, 2.4

* Model 2800, F. R. Hormann, Inc.

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mmoles $MgCl_2$, 1.8 mmoles K_2HAsO_4, 30.0 mmoles $NaHCO_3$, 60 μmoles DPN, 240 μmoles ATP, 75 ml bacterial extract and 40 ml muscle extract in a total volume of 350 ml was incubated at 37° for 3 h with continuous flow of moistened 95% N_2–5% CO_2 through the reaction flask into the $NaHSO_3$ tower. The reaction vessel was opened briefly at the start and the termination of the incubation for removal of samples for deoxyribose analysis. At the end of the incubation period a silicone antifoam agent was added and the reaction vessel was heated to 90° with vigorous gassing to drive off any remaining acetaldehyde. The acetaldehyde–bisulfite complex was oxidized with dilute acid dichromate at 60° and the acetic acid collected by steam distillation and titrated*.

The fermentation mixture was chilled, acidified to pH 1.0 with concentrated $HClO_4$, neutralized to pH 7.0 with KOH, filtered and the filtrate reduced to about 75 ml over a steam bath. The solution was acidified to congo red and extracted with ether for 36 h in a continuous extraction apparatus. The ether extract was neutralized and evaporated to dryness, a small amount of water was added, the solution was acidified and placed on a celite column for separation of the fermentation acids. The individual eluate fractions were neutralized and the fractions containing lactic acid were combined. After separation of the organic and aqueous phases, the sodium lactate solution was passed over a column of Dowex 50 (H^+ form) overlying a bed of 200 mg of charcoal. The effluent solution was titrated to pH 7.0 with standard NaOH and reduced in volume over a steam bath.

The acetate prepared from acetaldehyde and the lactate were degraded chemically to obtain radioactivity distribution patterns. Acetate was degraded with sodium azide using the method of PHARES[13]. Lactate was degraded to acetaldehyde and CO_2 by manganese dioxide as described by FRIEDEMANN AND GRAESER[9] and the acetaldehyde

TABLE I

PRODUCTS OF FERMENTATION OF THYMIDINE AND DR-5-P BY COMBINED EXTRACTS OF *E. coli* AND RABBIT MUSCLE

Additions	*Substrate*	*Thymidine 1.5 mmoles*	*None*	*DR-5-P 3.0 mmoles*
	Coli and muscle enzymes	+	+	+
Products Determined (mmoles)				
Acetaldehyde (iodimetric)		0.58	0.10	2.2
Acetate from acetaldehyde		0.54	0.00	2.1
Lactate from reaction mixture		0.82	0.12	2.7
Acetate from reaction mixture		0.05	0	0.4
"Formate" from reaction mixture		0	0	0.14
Deoxyribose degraded mmoles		1.10	—	2.94

The reaction mixtures contained substrates and enzymes as indicated above and other reagents in the amounts indicated in the text. When thymidine was used, phosphate buffer pH 7.0, 450 μmoles and when DR-5-P was fermented K_2HAsO_4, 1.8 mmoles were added. When thymidine was present and no enzymes were added, none of the products listed were produced.

* Many details of the procedures have been omitted, since many of the methods were adapted from procedures described by BERNSTEIN AND WOOD[12]. The "acid dichromate" refers to the solution described by these authors for the oxidation of ethanol.

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so obtained was oxidized to acetate and degraded as described above. Total combustions were done on DR-5-P, lactate and acetate by the VAN SLYKE-FOLCH method[14]. The formation of acetaldehyde and lactate from endogenous sources was determined by preparing incubation mixtures in which either substrate or enzyme extracts were omitted (Table I). In other experiments, thymidine was used as substrate for the fermentation procedure described in detail above except that phosphate was used instead of arsenate.

Chemical degradation of deoxyribose

As a check on the validity of the enzymic degradation, a partial chemical degradation was performed on two samples of deoxyribose. The degradative procedure used is summarized in Fig. 2.

The radioactive sample in the form of deoxyadenylic acid was diluted with a known amount of non-radioactive nucleotide, $MgCl_2$ was added and the phosphate ester was hydrolyzed with the 5'-nucleotidase present in crude snake venom. When hydrolysis was complete, 2.5 mmoles of deoxyadenosine were added. The nucleoside was hydrolyzed and radioactive adenine was removed as already described. In this case the solutions were deionized by treatment with Dowex 50 H^+ and Duolite A-4 OH^- and after filtration over small beds of charcoal the solutions were concentrated under reduced pressure in a rotary evaporator. Aliquots were removed for deoxyribose analysis and for total combustion and quantities between 1.5 and 2.0 mmoles were then taken for further treatment.

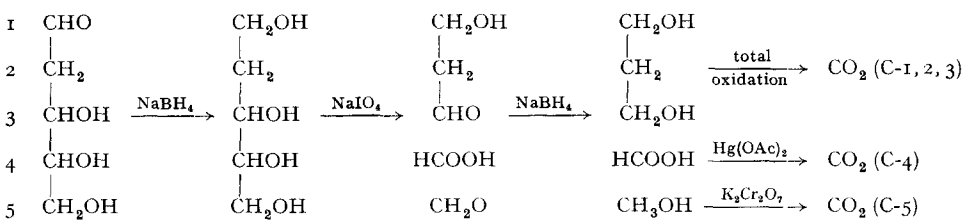

Fig. 2. Partial chemical degradation of deoxyribose.

Reduction of deoxyribose

To each sample of deoxyribose in 85 to 90 ml volume were added 275 mg $NaBH_4$ (7.25 mmoles) and the solution maintained at pH 8 to 9 by addition of 0.5 *N* H_2SO_4 until the rate of increase of pH became slow. The solutions were kept at room temperature for 1 ½ h, an additional quantity of 75 mg $NaBH_4$ was added to each, and after standing at room temperature for 45 min the solutions were stored in a refrigerator. The following day 0.5 *N* H_2SO_4 was added to adjust the solutions to pH 3, and the solutions again stored in the refrigerator to complete the destruction of excess $NaBH_4$. Colorimetric analysis showed that no deoxyribose remained. The product of the reduction is presumed to be the corresponding "deoxyribitol" (D-*erythro-2-deoxy*pentitol).

Oxidation of deoxyribitol

The deoxyribitol was not isolated, but was oxidized directly with $NaIO_4$. Control experiments showed that 2 moles of $NaIO_4$ were consumed per mole of compound,

forming formaldehyde, formic acid and another product which should be β-hydroxy propionaldehyde. Excess $NaIO_4$ (5.5 mmoles) was added to each sample and aliquots removed at 1½ and 2½ h for determination of unused oxidant[15]. Periodate consumption had ended by the time the first sample was taken.

Separation of the oxidation products

Since β-hydroxypropionaldehyde has been reported to decompose and polymerize during heating[16] it seemed inadvisable to attempt to remove formaldehyde and formic acid from the oxidation mixture by distillation. Instead, as soon as the iodimetric titration showed that oxidation was complete, the oxidation mixtures were chilled, a few drops of phenol red were added, the solutions neutralized with NaOH, and a large excess of $NaBH_4$ (3.0 grams) added*. As a result the formaldehyde is reduced to methanol and the β-hydroxypropionaldehyde to trimethylene glycol (propanediol-1, 3), while formate is unaffected. The mixtures were stoppered and stored in a refrigerator. Methanol was separated by distillation of ⅔ of the volume into a receiver chilled in ice. The residue in the distillation flask was made acid to congo red paper by addition of 12 *N* H_2SO_4, and transferred to a steam still for the distillation of formic acid. The residue of this distillation was deionized by the technique described for the deoxyribose solution, the effluent solution was concentrated, boric acid filtered out and the trimethylene glycol present in the filtrate was oxidized to CO_2. The formic acid was converted to CO_2 by the method of PIRIE[17]. The methanol solution was transferred to a 3-necked flask such as was used for decarboxylation of lactic acid[12] and the flask attached to the degradation train. The solution was chilled in ice, acidified and freed of extraneous CO_2. Oxidation of the methanol to CO_2 was then accomplished by addition of excess acid dichromate solution followed by a 30-min period of heating under reflux**.

RESULTS AND DISCUSSION

Data from typical fermentations of DR-5-P and thymidine, using combined extracts, are shown in Table I. When thymidine is used as substrate, about 70–80% of the sugar disappears. Recovery of acetaldehyde and lactate was about 0.5 moles and 0.3 moles respectively per mole of thymidine degraded. Yields of these products were considerably greater when DR-5-P was used as substrate. A possible explanation is that thymidine phosphorylase may be less stable than other enzymes in the *E. coli* extract, since several preparations were able to degrade DR-5-P but not thymidine. Also it is possible that the phosphodeoxyribomutase activity of the bacterial extract is low[2]. However no further experiments were carried out to explain the difference between the thymidine and DR-5-P fermentations. Table I also shows that the production of acetaldehyde, acetate and lactate from substrates present in the enzyme extracts is negligible. There is no formation of these substances in the absence of the enzyme extracts.

* We have observed that $NaBH_4$ reduces periodate, iodate and iodine instantaneously to iodide. This procedure avoids the exposure of aldehydes to iodine.

** See footnote page 127.

*** In control experiments 1.5 mmoles of known [6-^{14}C]glucose were oxidized with $NaIO_4$, excess $NaBH_4$ was added and the methanol and formic acid distilled and oxidized as above. Oxidation of the methanol yielded 1.5 mmoles of CO_2 of the expected radioactivity, while the CO_2 obtained from the formic acid contained no radioactivity.

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In the earliest experiments the enzymic degradation was carried out in two separate steps. In the first step, thymidine was treated with the bacterial extract to produce acetaldehyde and triose phosphate, the former being removed from the solution as already described. The "triose phosphate" solution was then concentrated and treated with muscle extract. The yields of acetaldehyde and lactic acid obtained by this procedure were poor and in all subsequent experiments, both extracts have been present together.

The recovery of acidic products derived from the fermentation of DR-5-P is shown in Table II. The amounts of "formate" and acetate isolated by chromatography are small. The acetate accounts for no more than 10–15% of the expected yield of 2-carbon fragments. In some experiments this acetate has been degraded and has been found to contain some radioactivity, indicating that it probably arose by enzymic action on small amounts of the intermediate fermentation products. Because the yields of acetaldehyde have been consistently high, the acetate obtained from the celite column has not been used to determine the isotope distribution in deoxyribose. The amounts of "formic acid" obtained have always been very small, and none of the samples have been degraded for isotope determination.

TABLE II

CHROMATOGRAPHY OF FERMENTATION ACIDS

Acid	*Peak effluent volume, ml*	*mmoles acid recovered*
Acetic	270	0.31
"Formic"	470	0.08
Lactic	540	2.52

The ether-extracted acids from the fermentation of 3.2 mmoles of DR-5-P were placed on a 25-g column of celite in a tube of 16 mm diameter. The column was then eluted with 150-ml portions of the following solvents: $CHCl_3$; 2.5% butanol–97.5% $CHCl_3$; 5% butanol–95% $CHCl_3$ and finally with 250 ml of 10% butanol–90% $CHCl_3$. The eluate was collected in 11-ml fractions and titrated. The acidic substances listed as "formic" acid have never been positively identified as such. On the type of column used formic and pyruvic acids have a peak effluent volume in this region of the chromatogram.

The formation of acetaldehyde from C-1 and C-2 and of lactic acid from C-3, C-4 and C-5 of deoxyribose is to be expected from the known enzymic activity of the two extracts. Table III shows the isotope distribution in two samples of deoxyribose of *E. coli* deoxyribonucleic acids. In sample 1, the high specific activity of C-1, C-2 and C-3, compared with the low specific activities in C-4 and C-5 shows that C-1, C-2 and C-3 do not contribute radioactivity to C-4 and C-5. Sample 2 shows that C-5 does not contribute appreciable amounts of radioactivity to C-2,3,4. The activity present in C-1 is probably not due to cross contamination, since the most likely route for cross contamination would involve the formation of acetaldehyde from the α and β carbons of the intermediate pyruvate, and cross contamination from C-5 would appear in C-2 of the sugar.

The possibility that radioactive contamination of the acetaldehyde (C-1 and C-2) might result from decarboxylation of pyruvate was tested in an experiment using [2-^{14}C]pyruvate in a reaction mixture containing the extracts and other reagents listed

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in Table IV in the same relative concentrations as were used for the fermentation of DR-5-P. Fructose-1,6-diphosphate was added in one reaction mixture to provide a source of triose phosphate, thus simulating the conditions of the deoxyribose fermentation. The amounts of acetaldehyde recovered were very small and actually were within the limit of error of the method. The conversion of [2-^{14}C]pyruvate to acetaldehyde was not more than 1.1% and since it is unlikely that the total amount of free pyruvate present at any time during a fermentation would be as high as the 0.5 to 1.0 mmole added in these experiments, the amount of pyruvate converted to acetaldehyde shown here is undoubtedly an overestimation.

TABLE III

ISOTOPE DISTRIBUTION* IN DEOXYRIBOSE OF *E. coli* GROWN IN ^{14}C SUBSTRATES

Carbon Atom	*Sample 1*	*Sample 2*
1	23.8	11.8
2	50.0	3.7
3	100.0	1.0
4	0.9	1.7
5	2.5	100.0

* Relative specific activity: for each sugar the carbon atom with the highest specific activity is assigned the arbitrary value of 100.

Sample 1 was isolated from cells in which the tracer was $CH_3{}^{14}COOH$, and sample 2 from cells grown in acetate with a tracer amount of [6-^{14}C]glucose added during the logarithmic phase of growth.

TABLE IV

TEST OF CONVERSION OF [2-^{14}C]PYRUVATE TO ACETALDEHYDE

Addition	*I*	*II*	*III*	*IV*
Fructose diphosphate (mmoles)	1.3	—	—	—
Na pyruvate (mmoles)	0.5	1.0	—	1.0
Na [2-^{14}C]pyruvate (counts/min)	$1.1 \cdot 10^6$	$1.1 \cdot 10^6$	—	$1.1 \cdot 10^6$
Bacterial extract (ml)	50.0	50.0	50.0	—
Muscle extract (ml)	15.0	15.0	15.0	—
Acetaldehyde recovered (mmoles) (Iodimetric estimation)	0.050	0.031	0.016	0.006
Lactate recovered mmoles	—	—	0.0	0.0
Total radioactivity in acetaldehyde (counts/min)	4,560	11,530	—	0.0
Radioactivity of acetaldehyde as % of total radioactivity added	0.4%	1.05%	—	—

All vessels contained 15.0 mmoles $NaHCO_3$, 1.2 mmoles $MgCl_2$, 0.9 mmoles K_2HAsO_4, 30 μmoles DPN, 126 μmoles ATP and other reagents noted in the table in a total volume of 200 ml. Reaction mixtures were swept with 95% N_2–5% CO_2 and the acetaldehyde collected in $NaHSO_3$ and oxidized to acetic acid. 1.0 mmole of carrier acetate was added and the acetic acid purified by steam distillation and converted to CO_2 for determination of radioactivity. Fermentation acids were isolated from the reaction mixtures by the method of ether extraction and elution from celite columns.

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In Table V, data from the enzymic and partial chemical degradations of two other samples of deoxyribose are compared. The percentage of isotope found in various parts of deoxyribose molecule by the different methods agree very closely. These findings confirm the lack of cross-over of isotope in the enzymic degradation.

The significance of the isotope distribution found under various growth conditions and with different tracers will be discussed in separate publications[18].

TABLE V

COMPARISON OF CHEMICAL AND ENZYMIC DEGRADATIONS

C-Atom	Sample 3			Sample 4		
	Enzymic		Chemical	Enzymic		Chemical
	Relative specific activity	Per cent of total radioactivity	Per cent of total radioactivity	Relative specific activity	Per cent of total radioactivity	Per cent of total radioactivity
1	23.8	97.0	95.7	3.7	3.8	3.5
2	45.9			0.3		
3	100.0			0.0		
4	1.8	1.0	2.4	1.6	1.5	1.5
5	3.5	2.0	1.8	100.0	95.3	94.9

Sample 3 was obtained from *E. coli* grown in [1-^{14}C]acetate and sample 4 from bacteriophage of *E. coli* produced in [6-^{14}C]glucose.

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REFERENCES

1 F. K. BAGATELL, E. M. WRIGHT AND H. Z. SABLE, *Biochim. Biophys. Acta*, 28 (1958) 216; *Federation Proc.*, 17 (1958) 184.
2 C. E. HOFFMAN AND J. O. LAMPEN, *J. Biol. Chem.*, 198 (1952) 885.
3 E. RACKER, *J. Biol. Chem.*, 196 (1952) 347.
4 I. A. BERNSTEIN AND D. SWEET, *Federation Proc.*, 16 (1957) 153.
5 E. VOLKIN, J. X. KHYM AND W. E. COHN, *J. Am. Chem. Soc.*, 73 (1951) 1533.
6 J. F. TAYLOR, A. A. GREEN AND G. T. CORI, *J. Biol. Chem.*, 173 (1948) 591.
7 P. K. STUMPF, *J. Biol. Chem.*, 169 (1947) 367.
8 Z. DISCHE, in *The Nucleic Acids*, Vol. I, p. 285, Academic Press, Inc., New York, 1955.
9 T. E. FRIEDEMANN AND J. B. GRAESER, *J. Biol. Chem.*, 100 (1933) 291.
10 E. BUEDING AND H. W. YALE, *J. Biol. Chem.*, 193 (1951) 411.
11 S. B. BARKER AND W. H. SUMMERSON, *J. Biol. Chem.*, 138 (1941) 535.
12 I. A. BERNSTEIN AND H. G. WOOD, in *Methods in Enzymology*, Vol. IV, p. 561, Academic Press, Inc., New York 1957.
13 E. F. PHARES, *Arch. Biochem.*, 33 (1951) 173.
14 D. D. VAN SLYKE AND J. FOLCH, *J. Biol. Chem.*, 136 (1940) 509.
15 E. L. JACKSON, *Organic Reactions*, II (1944) 341.
16 M. BERGMANN, A. MIEKELEY AND E. VON LIPPMANN, *Ber.*, 62 (1929) 1467.
17 N. PIRIE, *Biochem. J.*, 40 (1946) 100.
18 H. Z. SABLE, F. K. BAGATELL, E. M. WRIGHT AND J. SPIZIZEN, manuscripts in preparation.