Microbial Metabolism of 2-Deoxyglucose; 2-Deoxygluconic Acid Dehydrogenase*

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ABSTRACT: The intermediary metabolism of 2-deoxygluconic acid by cell-free extracts of a pseudomonad which utilizes 2-deoxyglucose as a carbon source was investigated. An NAD-dependent 2-deoxygluconate dehydrogenase has been characterized. The partially purified dehydrogenase was found to have a *p*H optimum of approximately 8; the equilibrium constant was calculated to be 3.4×10^{-12}. The K_m values for 2-deoxygluconate ranged from 1.54 to 5×10^{-2} M. The product of the enzymatic oxidation of 2-deoxygluconate has been characterized as a β-keto acid; it is proposed that the product is 2-deoxy-3-ketogluconate.

Until the present study was initiated, interest in the role of 2-deoxy-D-glucose in metabolic systems was concentrated for the most part on the activity of this compound as an antimetabolite. 2-Deoxy-D-glucose is known to be inhibitory to the normal activities and development of intact cells and organisms. Investigations on isolated enzyme systems have revealed that there are several sites at which 2-deoxy-D-glucose or its metabolites may be inhibitory. The following observations have been invoked as explanations of the inhibition caused by 2-deoxy-D-glucose: (1) The phosphorylation of 2-deoxy-D-glucose by hexokinase (Sols and Crane, 1954) and subsequent accumulation of 2-deoxy-D-glucose-6-phosphate deplete the ATP reserve of the cell. (2) 2-Deoxy-D-glucose-6-phosphate inhibits the oxidation of D-glucose-6-phosphate by glucose-6-phosphate dehydrogenase (Barban and Schulze, 1961). (3) The phosphohexoseisomerase reaction is inhibited by 2-deoxy-D-glucose-6-phosphate (Wick *et al.*, 1957). In addition, 2-deoxy-D-glucose is oxidized by glucose oxidase to 2-deoxy-D-gluconate (Sols and de la Fuenta, 1957; McComb *et al.*, 1957); it is not certain if this latter reaction contributes to the metabolic inhibition produced by 2-deoxy-D-glucose.

Although utilization of 2-deoxy-D-glucose for growth by fungi has been observed (Barnard and Challenger, 1949; Sols *et al.*, 1960), little information on the intermediary metabolism in these systems is available. Therefore it was of interest to elucidate the pathway of metabolism of 2-deoxy-D-glucose in an organism which could grow on this sugar. Furthermore, it was hoped that a study of the intermediary metabolism of 2-deoxy-D-glucose might contribute to the understanding of the mechanism of inhibition by this compound. A pseudomonad capable of utilizing 2-deoxy-D-glucose as a sole carbon source was employed in this investigation. The oxidation of 2-deoxy-D-glucose to 2-deoxy-D-gluconic acid and the subsequent conversion of the latter to a reducing compound by cell-free extracts of this bacterium have been reported (Eichhorn and Cynkin, 1963, 1964a). The NAD-dependent dehydrogenation of 2-deoxy-D-gluconate to 2-deoxy-3-ketogluconate is the subject of this report. A preliminary report of the present work has been presented (Eichhorn and Cynkin, 1964b).

Experimental Procedure

Materials. 2-Deoxy-D-glucose, D-glyceraldehyde, nucleotides, and coenzymes were purchased from the Sigma Chemical Co. Malonic semialdehyde was generated from ethyl β,β-diethoxypropionate, which was a gift from Dr. Minor J. Coon, by the method of Robinson and Coon (1962). The calcium salt of 2-deoxy-D-gluconic acid was prepared from 2-deoxy-D-glucose by a modification of the method of Hudson and Isbell (1929), and was converted to the potassium salt by treatment with Dowex 50 (K^+).

Cultivation of Bacteria. The microorganism used in these studies was a pseudomonad[1] which was isolated from soil by selective enrichment on the basis of its ability to utilize 2-deoxy-D-glucose as its only carbon source. The bacteria were grown with aeration at 25° for 72 hours in a minimal medium containing, in mmoles/liter: KH_2PO_4, 27.5; Na_2HPO_4, approximately 17.5; $MgSO_4$, 1.6; $CaCl_2$, 0.2; $FeCl_3$, 0.02; $MnCl_2$, 0.015; Na_2MoO_4, 0.005; and NH_4NO_3, 2.0. 2-Deoxy-D-glucose (0.25%) was added as a carbon source. The bacteria were harvested and washed once with a buffer consisting of 0.02 M potassium phosphate, 0.004 M $MgCl_2$, and 0.001 M cysteine hydrochloride, adjusted to *p*H 7. This will be referred to as buffer A.

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[1] For the purpose of reference, this microorganism will be designated *Pseudomonas* DG.

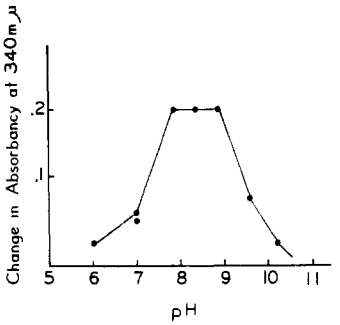


FIGURE 1: Effect of *p*H on reaction rate. Cuvets contained, in a volume of 1 ml, 5 μmoles of potassium 2-deoxygluconate, 2.5 μmoles of NAD, 0.08 mg of protein (fraction 2), and 120 μmoles of buffer as follows: potassium phosphate buffer (*p*H 6); potassium phosphate or Tris-HCl buffer (*p*H 7); Tris-HCl buffer (*p*H 8); Tris-HCl or sodium glycinate (*p*H 9); sodium glycinate (*p*H 9.7 and 10.5). The reaction was started by the addition of potassium 2-deoxygluconate. Incubation was carried out at 35°.

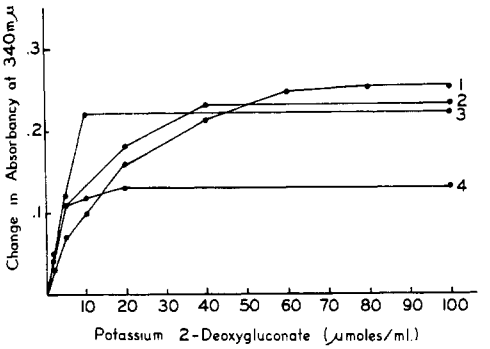


FIGURE 2: Effect of substrate concentration on reaction rate. Each cuvet contained, in a volume of 1 ml, 120 μmoles of sodium glycinate buffer (*p*H 9); NAD as follows: curve 1, 1.25 μmoles; curve 2, 2.5 μmoles; curve 3, 5 μmoles; curve 4, 10 μmoles; and potassium 2-deoxy-D-gluconate as indicated. The reaction was started by the addition of 5 units of enzyme. The absorbancy change reported is that which occurred between 1 and 5 minutes after the addition of enzyme. (A unit of enzyme is defined as the amount required for the reduction of 1 mμmole of NAD per minute when the respective concentrations of 2-deoxy-D-gluconate and NAD are 10 and 2.5 μmoles/ml.

Methods

Chromatography. The solvents employed for descending paper chromatography were as follows: solvent 1, pyridine-butanol-water (4:6:3); solvent 2, methyl ethyl ketone–water saturated with boric acid–glacial acetic acid (9:1:1); solvent 3, 1-butanol–glacial acetic acid–water (4:1:5) (the organic phase is used). Carbohydrates were detected on paper by the silver nitrate dip method of Trevelyan *et al.* (1950). Silica gel G was used as the adsorbent for thin-layer chromatography. The solvents used were solvent 4, toluene–ethyl acetate (7:3), and solvent 5, chloroform–ethyl acetate (7:3).

Assays. Reducing compounds were determined by the method of Nelson (1944) using D-glyceraldehyde as a standard. Protein was determined by the method of Lowry *et al.* (1951) using crystalline bovine serum albumin as a standard. The method developed by Kalnitsky and Tapley (1958) for the determination of oxalacetate and acetoacetate was used for the estimation of β-keto acids. Malonic semialdehyde was used as a standard. Malonic semialdehyde was itself standardized by the use of an NAD-linked malonic semialdehyde reductase which is present in extracts of *Pseudomonas* DG. β-Keto acids were also measured by the method described by Sistrom and Stanier (1953).

During the purification procedure, the enzymatic activity was determined as follows: Potassium 2-deoxy-D-gluconate (5 μmoles), 1.25 μmoles of NAD, 0.5 μmole of FMN, and 25 μmoles of potassium phosphate buffer (*p*H 7.1) were incubated in a total volume of 1 ml with a limiting amount of enzyme. The tubes were incubated at 30° for 2 hours; the reaction was stopped by boiling for 5 minutes, and the amounts of reducing compound in the deproteinized supernatants were determined with D-glyceraldehyde as a standard.

Although NAD was required for the oxidation of 2-deoxy-D-gluconate, the reduction of NAD in the presence of 2-deoxy-D-gluconate and crude extract could not be utilized as an assay procedure because of the presence of a potent $NADH_2$ oxidase. The purification procedure described below resolved $NADH_2$ oxidase activity from the 2-deoxy-D-gluconate dehydrogenase activity, and thus permitted the use of spectrophotometric assays in further studies of the properties of the enzyme.

The reduction of nicotinamide nucleotides was measured by following the increase in absorption at 340 mμ using a Gilford modification of the Beckman DU spectrophotometer. A Cary Model 14 spectrophotometer was used to observe spectral changes in experiments in which FMN and nicotinamide nucleotides were present together.

Results

Purification of the Enzyme. For the procedure given below, 5 g (wet wt) of bacteria, harvested from a 2-liter culture, was used. For larger-scale preparations, which were carried out in several instances, the amounts of all materials used were increased in proportion to the wet weight of the cells. All operations were carried out at 0–5°.

Preparation of Crude Extract. The harvested bacteria were suspended in buffer A to a total volume of 20 ml. The cells were broken by passage of the suspension through a French press at 19,200 psi. The crude extract was centrifuged at 100,000 × *g* for 1 hour; the pellet was washed with 8 ml of buffer A, and centrifuged

again at 100,000 × *g*. The wash was added to the 100,000 × *g* supernatant; the resulting solution (fraction 1) contained 7.5 mg of protein per ml.

Manganese Chloride Precipitation. Fraction 1 was diluted with an equal volume of buffer A, and 5 ml of 1 M $MnCl_2$ was added with stirring. The suspension was kept at 0° for 10 minutes, and then centrifuged at 10,000 × *g* for 20 minutes. The precipitate was discarded.

Ammonium Sulfate Fractionation. Solid ammonium sulfate (12.4 g) was added, with stirring, to the supernatant (38 ml) from the manganese chloride treatment to bring the solution to 55% saturation with respect to ammonium sulfate. After standing for 20 minutes at 0°, the suspension was centrifuged for 20 minutes at 10,000 × *g*. The supernatant was discarded and the precipitate was dissolved in 5 ml of 0.05 M Tris-HCl buffer (*p*H 7.1). The solution was dialyzed for 1 hour against 1 liter of 0.05 M Tris-HCl buffer (*p*H 7.1), and then for 12 hours against a second liter of the same buffer. The dialysate was centrifuged for 20 minutes at 10,000 × *g*, and the precipitate was discarded. The supernatant, which contained 2.5 mg of protein per ml (protein concentrations from various preparations ranged from 1.1 to 4.5 mg of protein per ml), will be referred to as fraction 2. The ammonium sulfate fractionation step resulted in a 3-fold purification of the enzymatic activity from the 100,000 × *g* supernatant; approximately 62% of the activity was recovered.

Stability of the Enzymatic Activity. Within 24 hours after the preparation of fraction 2, a loss of about one-third of the activity was usually observed. The remaining activity was stable for as long as 10 days when stored at −5°. The initial loss of activity was not prevented by the addition of cysteine (10^{-3} M), 2-mercaptoethanol (10^{-3} M), bovine serum albumin (5 mg/ml), or NAD (10^{-3} M).

*p*H *Optimum.* The *p*H optimum for the 2-deoxy-D-gluconate dehydrogenase reaction was found to be between 7.85 and 8.85 (Figure 1).

Affinity Constants. Attempts to determine the Michaelis-Menten constant (K_m) for 2-deoxygluconate were complicated by the inhibitory effects of NAD. In Figure 2 are shown the results of four experiments in which the rate of oxidation of 2-deoxygluconate was plotted as a function of 2-deoxygluconate concentration at different concentrations of NAD. At the highest concentration of NAD tested, 10 mM, curve 4 demonstrates the inhibitory effect of this concentration. Consequently, no single K_m value could be obtained. By plotting the data in Figure 2 according to the method of Lineweaver and Burk (1934), K_m values were obtained which ranged from 1.54 to 5.0 × 10^{-2} M. The K_m for NAD was not obtained.

Characteristics and Requirements of the Reactions. It had been observed that incubation of 2-deoxy-D-gluconate with cell-free extracts in the presence of NAD resulted in the production of a reducing compound. Furthermore, it was found that the amount of reducing compound formed was increased by the addition of FMN to the reaction mixture. The effect of several other cofactors on the reaction was negligible; these results are shown in Table I.[2] The addition of the following cations had no effect on the reaction: Mg^{2+}, Mn^{2+}, Zn^{2+}, Co^{2+}, Fe^{3+}, and Na^{+}. The rate of oxidation of 2-deoxy-D-gluconate was increased, and the steady-state concentration of $NADH_2$ was decreased by the addition of FMN to the incubation mixture.

TABLE I: Effect of Cofactors on Production of Reducing Compound.[a]

Expt	Tube	Time (hr)	Reducing Compound (μmoles)
1	Complete	0	3.1
	Complete	4	4.7
	− NAD, + NADP	4	3.1
	+ TPP,[b] + FAD	4	5.8
	+ TPP, + FMN	4	8.0
	+ FAD	4	5.8
	+ Cytochrome *c*	4	4.4
2	Complete	0	3.8
		0.5	5.3
		4	3.3
	+ FMN	0.5	6.7
		4	8.7
	+ FMN, + TPP	0.5	6.2
		4	8.4

[a] The complete system consisted of 10 μmoles of potassium 2-deoxygluconate, 2.5 μmoles of NAD, 50 μmoles of potassium phosphate buffer, *p*H 7.1, and 0.5 ml of enzyme (3000 × *g* supernatant). Cofactors were added where indicated in the following amounts: NADP, 2.5 μmoles; TPP, 2.5 μmoles; FAD, 1 μmole; FMN, 1 μmole; cytochrome *c*, 0.5 mg. Tubes were incubated at 25°, and the reaction was stopped by boiling for 5 minutes. [b] Thiamin pyrophosphate.

Fraction 2 was found to catalyze the oxidation of $NADH_2$ in the presence of FMN. Therefore, an incubation was carried out in which the reduction of both NAD and FMN was followed spectrophotometrically. To permit $FMNH_2$ to accumulate, the amount of available oxygen was made limiting by placing a layer of mineral oil over the cuvet contents. It was estimated that, under the experimental conditions, the dissolved oxygen (approximately 1 μmole) would be exhausted in 90 minutes.

The results of this experiment are shown in Figure 3. The observation that $FMNH_2$ accumulates as oxygen

[2] Relatively high zero-time readings in Tables I and II are owing to the reactivity of NAD in the Nelson test when present at high concentrations.

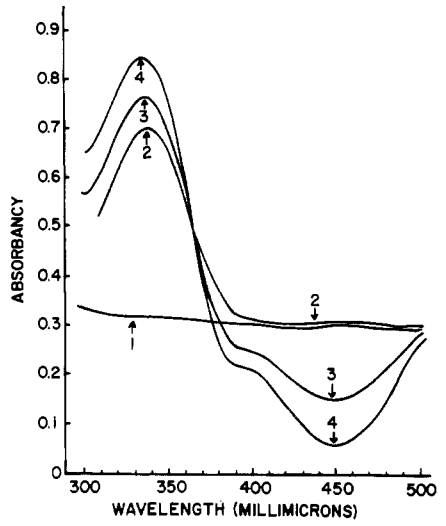
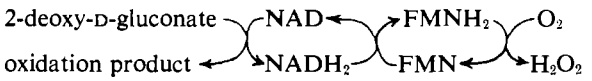


FIGURE 3: Reduction of FMN as oxygen becomes limiting. The experimental cuvet contained, in a volume of 1 ml, 5 μmoles of potassium 2-deoxygluconate, 0.04 μmole of FMN, 2.5 μmoles of NAD, 120 μmoles of sodium glycinate buffer (*p*H 9); and 0.1 ml of fraction 2. Mineral oil (0.2 ml) was placed over the cuvet contents. The reaction was started by the addition of potassium 2-deoxygluconate. Curve 1 represents the zero-time control; curves 2, 3, and 4 represent the spectra of the reaction mixture at 25, 90, and 120 minutes, respectively.

becomes exhausted suggests a sequential transfer of hydrogen in the following manner:



Subsequently it was found that the addition of acetaldehyde and alcohol dehydrogenase stimulated the production of reducing sugar at least as much as did FMN (see Table II). Presumably, either FMN or the alcohol dehydrogenase system increased the extent of oxidation of 2-deoxy-D-gluconate by displacing the equilibrium of the dehydrogenase reaction.

Equilibrium Constant. The stimulatory effects of FMN on the alcohol dehydrogenase system are reflected in the very unfavorable equilibrium constant of the reaction. From the data presented in Figure 4 the average equilibrium constant was calculated to be 3.4×10^{-12}.

Effect of Growth Conditions on Substrate and Cofactor Specificity. The data in Table III summarize the results of a comparison of the dehydrogenase activities of fraction 2 prepared from cells grown either on 2-deoxy-D-glucose or on D-glucose. It can be seen that, whereas cells grown on 2-deoxy-D-glucose contain a NAD-linked 2-deoxy-D-gluconate dehydrogenase, glucose-grown cells are completely devoid of this activity. This observation indicates that 2-deoxygluconate dehydrogenase is an inducible enzyme. On the other hand, the oxidative activity of these extracts toward gluconate displays a more complex pattern. Both NADP- and NAD-dependent gluconate dehydrogenase activities are present in both types of extracts. Although this activity has not been investigated in detail, the similar specific activities of the two extracts in the presence of NADP would indicate that NADP-dependent gluconate dehydrogenase is a constitutive enzyme. The 3-fold increase in NAD-linked gluconate oxidation in 2-

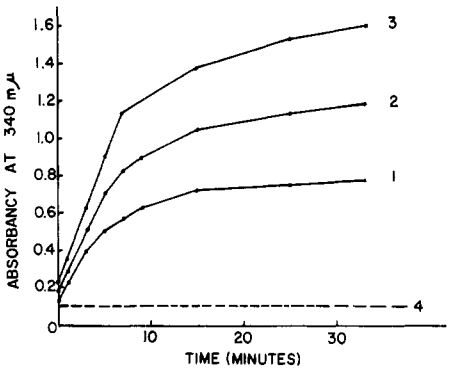


FIGURE 4: Reduction of NAD by 2-deoxygluconate and fraction 2. Each cuvet contained, in a volume of 1 ml, 5 μmoles of potassium 2-deoxygluconate, 120 μmoles of sodium glycinate buffer (*p*H 9), 0.1 ml of fraction 2, and NAD as follows: cuvet 1, 0.5 μmole; cuvet 2, 1.25 μmoles; cuvet 3, 2.5 μmoles. The reactions were started by the addition of potassium 2-deoxygluconate (omitted from cuvet 4).

TABLE II: Relative Effects of FMN and Alcohol Dehydrogenase System on Oxidation of 2-Deoxygluconate.[a]

Tube No.	Time (hr)	Reducing Sugar (μmoles/ml incubation supernatant)
1	0	3.3
2	0.33	6.6
3	1	9.7
4	4	11.4
5	0	3.4
6	4	7.8

[a] Each tube contained, in a volume of 4 ml, 20 μmoles of potassium 2-deoxygluconate, 5.0 μmoles of NAD, and 400 μmoles of glycine-NaOH buffer, *p*H 9. Thirty μmoles of acetaldehyde and 70 units of alcohol dehydrogenase were added to tubes 1–4. Two μmoles of FMN were added to tubes 5 and 6. Incubation was carried out at 30°; the reaction was stopped by boiling for 5 minutes.

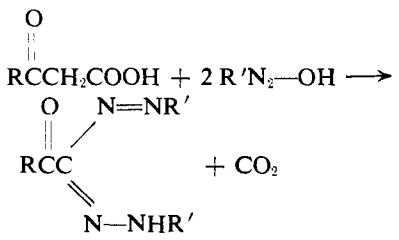
TABLE III: Comparison of Gluconate and 2-Deoxygluconate Dehydrogenase Activities in Fraction 2 from Cells Grown on Glucose and 2-Deoxyglucose.[a]

Cuvet No.	Growth Substrate	Pyridine Nucleotide	Aldonic Acid	mμMoles Pyridine Nucleotide Reduced per Minute per mg Protein
1	Glucose	NAD	Gluconate	2.33
2	Glucose	NADP	Gluconate	7.36
3	Glucose	NAD	2-Deoxygluconate	0
4	Glucose	NADP	2-Deoxygluconate	0
5	2-Deoxyglucose	NAD	Gluconate	8.00
6	2-Deoxyglucose	NADP	Gluconate	5.91
7	2-Deoxyglucose	NAD	2-Deoxygluconate	32.00
8	2-Deoxyglucose	NADP	2-Deoxygluconate	0

[a] Cuvets contained, in a volume of 1 ml, 5 μmoles of potassium gluconate or potassium 2-deoxygluconate, 2.5 μmoles of NAD or NADP, and 120 μmoles of sodium glycinate buffer (*p*H 9). Cuvets 1–4 contained 0.38 mg of protein; cuvets 5–8 contained 0.8 mg of protein. The reactions were started by the addition of the aldonic acid.

deoxygluconate-grown cells may be owing to the action of NAD-dependent 2-deoxygluconate dehydrogenase on gluconate.

Tentative Identification of Product as 2-Deoxy-3-ketogluconate. The product of the 2-deoxy-D-gluconate dehydrogenase reaction, on the basis of several criteria, is a β-keto acid, presumably 2-deoxy-3-ketogluconic acid. β-Keto acids are known to react with diazotized *p*-nitroaniline to produce a formazan derivative (Kalnitsky and Tapley, 1958) according to the following equation:



Treatment of unboiled reaction mixtures similar to those described in Table II (tube numbers 2–4) with diazotized *p*-nitroaniline resulted in the formation of a formazan derivative, which was distinguished from the derivatives of malonic semialdehyde and acetoacetate on thin-layer chromatography (solvents 4 and 5). An experiment was performed in which the reduction of NAD was followed for various times; at the end of these periods the amounts of β-keto acid present in the reaction mixtures were determined. The results are given in Table IV. It can be seen that there is a 1:1 relationship, on a molar basis, between the amount of NAD reduced and β-keto acid formed. Boiling the reaction mixtures prior to treatment with diazotized *p*-nitroaniline destroyed the reactivity in this assay. In an earlier communication (Eichhorn and Cynkin, 1964a), a product of 2-deoxygluconate metabolism was identified as glyceraldehyde on the basis of the melting point of its 2,4-dinitrophenylhydrazone and its chromatographic properties in solvents 1 and 3. However, the metabolic product reported in that study has a different mobility from glyceraldehyde in solvent 2 (R_F = 0.47 compared to 0.20 for glyceraldehyde). It now appears that this product is formed when the incubation mixtures are boiled. That is, the disappearance of 2-deoxy-3-ketogluconic acid upon boiling, as estimated by the loss of reactivity with diazotized *p*-nitroaniline, is accompanied by the appearance of a reducing compound with the properties ascribed to it previously. Although this compound has not been further characterized, it is suggested that it is 1-deoxyribulose, the ex-

TABLE IV: Stoichiometry between NAD Reduction and Formation of β-Keto Acid.[a]

Number	Time (min)	NADH (μmoles)	β-Keto Acid (μmoles)
1	0	0	0.005
2	5	0.059	0.055
3	10	0.114	0.110
4	30	0.230	0.170

[a] Each cuvet contained, in a volume of 1 ml, 5 μmoles of potassium 2-deoxygluconate, 2.5 μmoles of NAD, 120 μmoles of sodium glycinate buffer (*p*H 9), and 0.2 ml of fraction 2. The reaction was started by the addition of potassium 2-deoxygluconate.

pected decarboxylation product of 2-deoxy-3-keto-gluconate. It is interesting to note that when gluconate was oxidized in the presence of NAD and fraction 2 from 2-deoxy-D-glucose-grown cells, the incubation mixture contained a compound which, on the basis of its reactivity in the cysteine-carbazole reaction (Dische and Borenfreund, 1951) and chromatographic properties, appears to be ribulose, the expected decarboxylation product of 3-keto-D-gluconate.

Additional evidence for the β-keto acid structure was furnished by the observation that the product of the enzymatic reaction was decarboxylated by treatment with 4-aminoantipyrine, a reagent which is known to decarboxylate β-keto acids (Sistrom and Stanier, 1953). The details of this experiment are given in Table V. There is a 1:1 stoichiometry between the β-keto acid as determined by the reaction with diazotized *p*-nitroaniline and the CO_2 evolved by treatment with 4-aminoantipyrine.

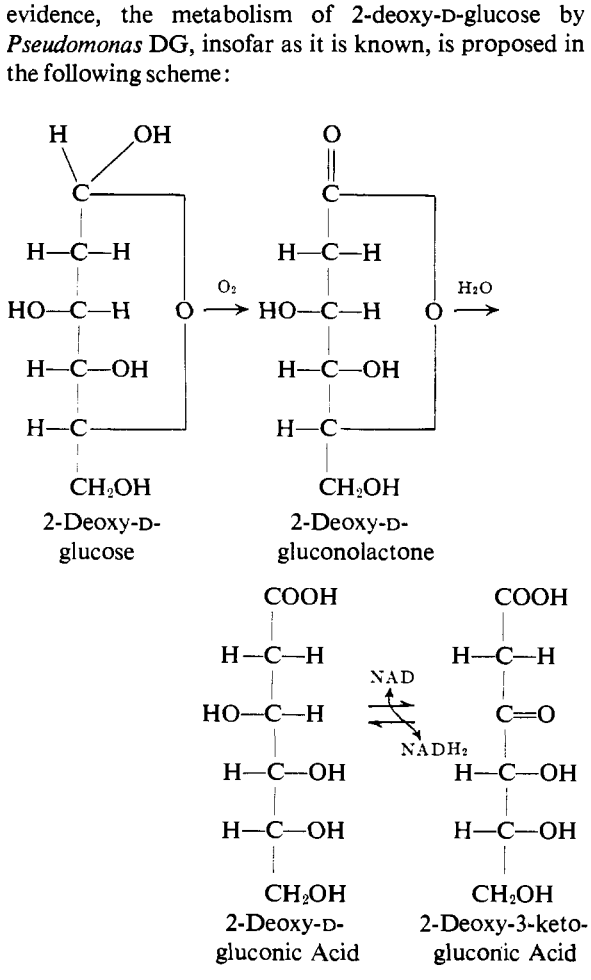
TABLE V: Decarboxylation with 4-Aminoantipyrine.[a]

Time (hr)	Formazan Derivative (μmoles/ml)	CO_2
0	0	0.15
4	1.60	1.55

[a] For this experiment, 40 μmoles of potassium 2-deoxygluconate, 10 μmoles of NAD, 64 μmoles of acetaldehyde, 225 units of alcohol dehydrogenase, and 800 μmoles of sodium glycinate buffer (*p*H 9) were incubated for 4 hours at 30°. At the end of the incubation period the tubes were placed in an ice bath. Treatment with 4-aminoantipyrine was carried out in Warburg flasks as follows: 2.25 ml from each tube was placed in the flasks with 0.75 ml of 2 M sodium acetate buffer (*p*H 3.8); 0.6 ml of 0.1 M 4-aminoantipyrine was placed in the side arm. After mixing, the CO_2 evolved was measured manometrically.

Discussion

Although the evidence for the identification of the compound produced by the enzymatic oxidation of 2-deoxy-D-gluconate is not unequivocal, its properties, and in particular its reactivity with diazotized *p*-nitroaniline and with 4-aminoantipyrine, are consistent with a 3-keto acid structure. On the basis of the available evidence, the metabolism of 2-deoxy-D-glucose by *Pseudomonas* DG, insofar as it is known, is proposed in the following scheme:



The oxidation of the nonphosphorylated sugar to the corresponding aldonic acid is consistent with the patterns observed in other pseudomonads for the metabolism of D-glucose (Wood, 1955), D-galactose (DeLey and Doudoroff, 1957), and D- and L-arabinose (Palleroni and Doudoroff, 1956; Weimberg and Doudoroff, 1955). The oxidation of 2-deoxy-D-gluconic acid to a 3-keto acid departs from these patterns, since in other pseudomonads the hydroxyl group at either carbon 2 or carbon 5 usually becomes oxidized (Wood, 1955) in the subsequent metabolism of nonphosphorylated aldonic acids. However, an analogous reaction in hog kidney has been reported by Smiley and Ashwell (1961), in which L-gulonic acid is oxidized by an NAD-dependent dehydrogenase to 3-keto-L-gulonic acid. Furthermore, in the hog kidney system, 3-keto-L-gulonic acid is enzymatically decarboxylated to form L-xylulose. In the present system, although the decarboxylation of 3-keto-2-deoxy-D-gluconic acid has not yet been shown to be enzymatically catalyzed, this would be one possible route for further metabolism.

Both the enzymatic system described in the present paper and L-gulonic dehydrogenase may be contrasted to 6-phosphogluconic dehydrogenase, which catalyzes the oxidative decarboxylation of 6-phosphogluconic acid to ribulose-5-phosphate; the presumed intermediate, 3-keto-6-phosphogluconic acid, has never been isolated and is thought to exist only as an enzyme-bound compound. The first instance of isolable 3-keto sugar derivatives occurring as intermediates in a metabolic pathway was reported by Bernaerts and DeLey (1958), in which lactose, maltose, and their corresponding bionic acids were oxidized by growing cells of a microorganism, *Agrobacterium tumefaciens* (first reported as *Alcaligenes*), to their appropriate glycosid-3-uloses. Subsequent studies revealed that trehalose and sucrose were similarly metabolized (Bernaerts and

De Ley, 1960a,b, 1961, 1963; Feingold *et al.*, 1961; Fukui and Hochster, 1963).

It is interesting to note, in light of current knowledge of the action of 2-deoxy-D-glucose as a metabolic inhibitor, that in *Pseudomonas* DG, 2-deoxy-D-glucose-6-phosphate does not appear to play a role in the metabolism of 2-deoxy-D-glucose. It would be of interest to observe in those organisms which have been reported to be "resistant" to 2-deoxy-D-glucose whether such organisms are able to metabolize this potential inhibitor by way of a nonphosphorylated pathway so that phosphorylated derivatives of 2-deoxy-D-glucose would not be available to interfere with the "normal" metabolism of hexose phosphates.

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