ENDOGENOUS RESPIRATION AND OXIDATIVE ASSIMILATION IN *NOCARDIA CORALLINA*

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Nocardia corallina oxidizes a variety of organic compounds including glucose, acetate, succinate, and propionate and, in each case, the amounts of oxygen used and carbon dioxide released have been less than the theoretical values for the complete oxidation of the substrate. The incubation systems contained no products of incomplete oxidation of the substrates and, with the results obtained from using uncoupling agents (sodium azide and 2,4-dinitrophenol), it appeared that the low gas exchange values were due to concurrent oxidative assimilation from the substrates.

Oxidative assimilation has been studied with many bacterial species and the subject has been reviewed by Clifton (1952, 1957). Barker (1936) studied the oxidation of substrates by the colorless alga *Prototheca zopfii*, and formulated the problem of correcting for endogenous respiration when studying microbial assimilation, manometrically. He stated that "one may take that method of correction to be most basically correct which when applied to all vessels gives the most constant value of oxygen consumption per unit quantity of substrate decomposed." The validity of subtracting endogenous values in calculating oxygen uptake figures for substrates removed by cells has been reviewed and discussed by Wilner and Clifton (1954). In general, the endogenous respiration does not appear to be suppressed during exogenous respiration (Moses and Syrett, 1955; Cochrane and Gibbs, 1951; Blumenthal *et al.*, 1951; Santer and Ajl, 1954).

This communication (a) considers the relationship between endogenous and exogenous respiration for *N. corallina*, (b) describes the effect of inhibitors on cellular respiration, and (c) includes evidence for oxidative assimilation from isotopically labeled substrates.

METHODS

The strain of *N. corallina* used in the investigation was isolated by enrichment culture using uracil as the main source of carbon and nitrogen (Batt and Woods, 1951). The parent strain was preserved as a freeze-dried preparation and, for experimental work, a stock culture was maintained on glucose (0.75 per cent)-yeast extract (10 per cent)-agar (3 per cent) slopes.

Preparation of cell suspensions. Growth media (200 ml/500 ml conical flask, pH 7.2), containing, per L, a carbon substrate e.g., glucose, acetate or propionate (2 g), ammonium sulfate (1.5 g), thiamine (1.0 mg), inorganic salts (KH_2PO4, 5 g; $MgSO_4 \cdot 7H_2O$, 2 g; and $CaCl_2$, 2 g) and phosphate buffer (0.1 M) were inoculated from 48-hr-old glucose-yeast extract-agar slopes and incubated aerobically at 30 C with slow shaking (60 oscillations per min). The cells were harvested by centrifugation (2500 × G), washed once with phosphate buffer (0.1 M; pH 7.2), and suspended in buffer to give a suspension equivalent to 10 mg dry weight cells per ml. The suspensions showed no detectable alteration in metabolic activity after storing for several days at 0 to 4 C.

Manometric experiments. (1) With C^{12}-substrates:—Cell suspension experiments were carried out in Warburg manometers using conventional procedures (Umbreit *et al.*, 1949) for estimating oxygen consumption and carbon dioxide production. Each flask contained cells (equivalent to 10 mg dry weight), phosphate buffer (0.066 M; pH 7.2), substrate (10 μmoles); total volume, 3.2 ml. The incubation temperature for all experiments was 30 C. The rates of oxygen consumption (Q_{O_2}) were expressed as microliters of oxygen consumed per hr per mg dry weight cells.

(2) With C^{14}-substrates:—Studies on cell suspensions oxidizing C^{14}-labeled substrates were based on an initial incubation of cells (150 dry weight) with C^{14}-labeled substrate (200 μmoles) in phosphate buffer (0.033 M; pH 7.2; 30 ml) using large Warburg manometer cups (volume, 210 ml). The oxidations proceeded

to completion and the rate and extent of the reactions were followed by measuring oxygen uptake. At the conclusion of the experiment, sulfuric acid (5.0 ml; 1.0 N) was tipped into the system from a side bulb and the flask left to shake for 30 min. The KOH (10 per cent) in the center well was removed and, together with the washings and paper, made up to a final volume of 5.0 ml with CO_2-free distilled water for plating. The incubation system was centrifuged to give a supernatant and cells, preparatory to wet combustion and plating.

(3) Release of radioactive CO_2 from C^{14}-labeled cells:—Labeled cells (C^{14}) were prepared by incubation of strain S (grown on a glucose ammonium sulfate-thiamine medium; 190 mg dry weight) with a C^{14}-labeled substrate (200 μmoles: either 3-C^{14}-propionate, 1-C^{14}-acetate, or U-C^{14}-glucose) until exogenous oxygen-uptake ceased. The cells were removed, washed and resuspended in phosphate buffer (pH 7.2; 0.033 M) to give a final concentration of cells equivalent to 10 mg dry weight/ml. The activity of the cells was determined by wet combustion of a sample followed by plating and counting of the CO_2 produced.

The release of $C^{14}O_2$ from the cells during exogenous and endogenous respiration was measured by incubating the cells (14 mg dry weight) in small manometer cups (volume, approximately 30 ml); half of the samples (7 manometers) in the absence of added substrate and the remainder (7 manometers) with 100 μmoles of unlabeled carbon substrate. At hourly intervals the reaction in a manometer from each group was stopped by tipping sulfuric acid (0.2 ml; 2 N) from a side bulb and, after shaking to permit dissolved CO_2 to be taken up by the center well KOH, the contents of the center well were removed for counting.

Radioactivity estimations. Samples containing carbon solely in the form of carbon dioxide were plated as $BaCO_3$ on filter paper discs and counted with an end window Geiger-Müller counter. The counts recorded were corrected for background and selfabsorption. The radioactivity in cells or supernatants from incubation systems was estimated by first oxidizing the organically bound carbon to carbon dioxide with a chromic acid solution (Van Slyke and Folch, 1940) and then plating the trapped carbon dioxide (in 3 N NaOH) as $BaCO_3$.

Chemicals. Sodium propionate (3-C^{14}) was supplied by Professor H. G. Wood; U-C^{14}-glucose and 1-C^{14}-acetate were obtained from the Radiochemical Centre, Amersham, England.

Fractionation of cellular constituents. Cells (290 mg dry weight) grown under standard conditions in a glucose-ammonium sulfate-thiamine medium for 48 hr were incubated with 3-C^{14}-propionate (220 μmoles) in a large Warburg flask until the oxidation of the propionate was complete. The cells were centrifuged, washed once with distilled water, and a sample oxidized to CO_2 to determine the extent of the assimilation from propionate (68,700 cpm in 290 mg wet weight cells). The residual cells were dried, ether extracted for 4 hr, and the ether solution evaporated to dryness; yield of lipid, 83.5 mg. The dried cells were next extracted for 2 hr with boiling chloroform (100 ml); yield of lipid, 15.1 mg. The ether and chloroform soluble components of the cells therefore constituted 33.8 per cent of the dry weight of the cells.

Polysaccharides were separated from the cells (now lipid-free) by treatment with 15 per cent KOH (50 ml; 100 C; 2 hr) and the supernatant, containing carbohydrate was prepared by centrifugation. The clear solution was neutralized with 5 N HCl and further hydrolysis carried out by adding an equal volume of 2 N HCl and heating (100 C; 1 hr). Glucose (10 mg; C^{12}) was added to the hydrolyzate which was extracted three times with small volumes (5.0 ml) of redistilled pyridine. The pyridine extracts were evaporated to dryness and the residue extracted with water (5.0 ml). To the aqueous solution, 2,4-dinitrophenylhydrazine hydrochloride (100 mg) in acetate buffer (5.0 ml; pH 5.0) was added and the mixture heated on a boiling water-bath for 1 hr. On cooling a crude crystalline osazone was obtained; yield, 51.2 mg. Two samples of the osazone (10 mg) were oxidized to CO_2 and assayed for C^{14} activity.

A sample of glucose (10 mg) subjected to the same procedures gave a good yield of glucosazone (18 mg).

RESULTS

Endogenous respiration of strain S. Factors affecting the endogenous respiration of strain S were studied, initially, by growing the organism on different carbon substrates and determining the respiratory activity of cell suspensions pre-

TABLE 1

Endogenous respiration and acetate oxidation by various cell suspensions of strain S

Carbon Substrate for Cell Growth	Growth Time	Endogenous Respiration Values		Respiration Values for Acetate Oxidation		μMoles O_2 Consumed/ μMole Acetate
		Q_{O_2}	RQ	Q_{O_2}	RQ	
	hr					
Acetate	24	12	0.92	69	0.94	0.89
	48	7	0.91	39	0.91	1.00
	72	1	1.00	17	0.97	0.99
	96	0	—*	16	0.91	1.01
Propionate	24	14	0.98	66	1.08	0.94
	48	11	0.89	34	0.93	0.94
	72	7	1.07	32	0.97	1.02
	96	0	0.97	13	0.99	1.03
Glucose	24	11	0.94	60	1.06	0.96
	48	15	1.09	38	0.90	0.98
	72	11	1.00	27	1.07	1.00
	96	4	1.00	14	1.00	1.00

* The respiration rate was too low to give a significant CO_2 production value.

The experiments were carried out using the standard manometric conditions (see Methods). Acetate concentration: 10 μmoles per manometer cup. The Q_{O_2} values were determined from the maximum gradient of the oxygen-uptake curves. The RQ figures were calculated from the total oxygen uptake and the CO_2 production values after 3 hr incubation, i. e., at a time when all of the substrate had been degraded by the cells.

pared from inoculated media which had been incubated aerobically at 30 C for either 24, 48, 72, or 96 hr. Each growth medium contained ammonium sulfate, thiamine, inorganic salts, and either acetate, propionate, or glucose as the carbon source (see Methods). The cell suspensions were studied manometrically to determine the rates of endogenous respiration ($Q_{O_2}{}^{E}$) and the respiratory quotients for the cells (table 1). The growth curves were determined for the organism growing in media containing the three different carbon substrates; growth was complete in 24, 48, and 72 hr for media containing, respectively, acetate, propionate, and glucose as the carbon substrates. Although it is convenient to refer to the period of incubation of the inoculated medium (i. e., prior to centrifuging for the preparation of cell suspensions) as the growth time for the cells, it bears no direct relationship to either the mean generation time for the organism or to the time required for maximum cell density to be reached in the growth medium. The growth time represents a period of active growth on the substrate and, for some of the longer growth times, a period (before harvesting of the cells) of incubation of the nonproliferating cells (i. e., in the "resting" state).

Although the rates of endogenous respiration for strain S (table 1) decreased with increasing growth times for the cells, the respiratory quotients for all of the suspensions tested were included in the range 0.89 to 1.09. The rate of endogenous respiration for each suspension was constant during the course of the manometry experiments, i. e., oxygen-uptake curves for all of the suspensions used were linear.

Effect of azide, iodoacetate, and arsenite on the endogenous respiration. With cells grown in a

TABLE 2

Effect of azide, iodoacetate, and arsenite on the rate of endogenous respiration

Additions to Incubation System*	Q_{O_2}	Per Cent Difference from Q_{O_2} = 16
Nil	16	0
Sodium azide		
0.0033 M	24	+50
0.0016 M	21	+31
0.0008 M	18	+12
0.0004 M	17	+6
Sodium iodoacetate		
0.001 M	12	−25
0.0005 M	13	−19
0.0002 M	14	−12
0.0001 M	15	−6
Sodium arsenite		
0.0033 M	9	−43
0.0016 M	9	−43
0.0008 M	14	−13
0.0004 M	16	0

* The incubation system contained cells (10 mg dry weight) in phosphate buffer (0.066 M; pH 7.2) and the inhibitors were added to give the final concentrations shown in the table. The Q_{O_2} values were determined for the interval 60 to 90 min; the effect of choosing another time interval during the manometry experiments was slight since the oxygen-uptake curves were essentially linear. Increasing the final concentrations of sodium iodoacetate and sodium arsenite above the values shown depressed the Q_{O_2} values further.

glucose-ammonium sulfate-thiamine medium for 48 hr, the rates of endogenous respiration were considerably increased in the presence of 0.0033 M and 0.0016 M sodium azide (table 2). The $Q_{O_2}{}^{E}$ values were (a) unaffected by azide concentrations lower than 0.0004 M and (b) reduced with azide at concentrations above 0.01 M. The oxygen-uptake curves were linear at each concentration of sodium azide tested.

Sodium iodoacetate and sodium arsenite were tested, each at several different concentrations (table 2). Both substances at the higher concentrations inhibited the rate of endogenous respiration but at no concentration tested was there any increase in the $Q_{O_2}{}^{E}$ values.

Oxidation of acetate by cell suspensions. Variations in the respiration values for cells of strain S oxidizing acetate were studied using suspensions of the organism (a) prepared after different growth times (24, 48, 72, or 96 hr) and (b) grown on different carbon substrates (acetate, propionate, or glucose). The respiration values obtained (table 1) were all corrected for the endogenous values, the validity of this procedure having been deduced first from experiments (reported below) on the release of C^{14} from labeled cells in the presence and absence of acetate and then from the observation that the ratios, μmoles O_2 consumed per μmole acetate oxidized, shown in table 1, were included in a range of 1.01 to 1.92 if no correction for endogenous respiration was made (range for corrected values, 0.89 to 1.03). The rates of oxidation of acetate were lower with cells reaped after the longer growth times, e. g., with propionate-grown cells, the $Q_{O_2}{}^{acetate}$ figures paralleled more closely the change in the $Q_{O_2}{}^{E}$ values for the cell suspensions. The relationship between the rates of (a) acetate oxidation and (b) endogenous respiration for cells grown on acetate as the carbon substrate are indicated in the corrected oxygen-uptake curves for acetate oxidation (figure 1).

Oxidation of propionate and glucose by cell suspensions. The cell suspensions used to study acetate oxidation (table 1) were similarly tested with propionate and glucose as substrates. The $Q_{O_2}{}^{propionate}$ and $Q_{O_2}{}^{glucose}$ values were lower with older cell suspensions; $Q_{O_2}{}^{propionate}$ for propionate-grown cells decreased from 90 for 24 hr cells to 14 for 96 hr cells and the $Q_{O_2}{}^{glucose}$ for glucose-grown cells decreased from 25 (24-hr cells) to 11 (96-hr cells). These decreases in Q_{O_2}

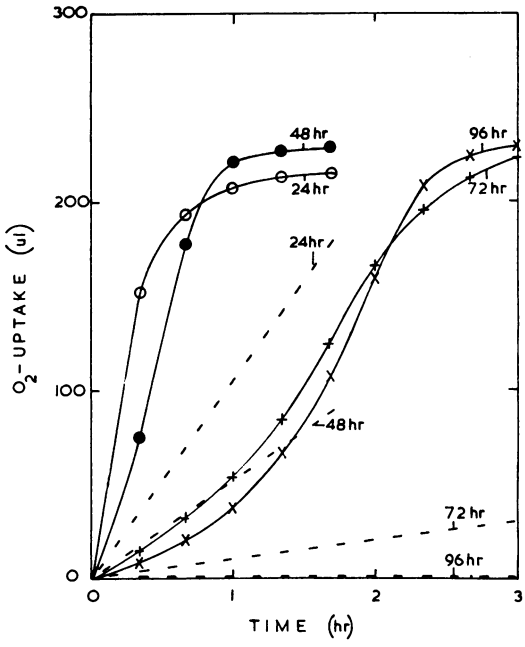

Figure 1. The oxygen uptake curves for cell suspensions grown for various times (shown on the graphs) in an acetate-ammonium sulfate-thiamine medium and incubated either with acetate (10 μmoles; *solid lines*) or alone (*broken lines*). The results for acetate oxidation have been corrected for endogenous respiration.

paralleled the observed decreases in the endogenous respiration rates of the respective cells. The respiratory quotients for propionate oxidation were all within the range 0.78 to 0.89 and the ratios, μmoles O_2 consumed per μmole propionate oxidized, were in the range 1.43 to 2.00. The corresponding figures for glucose oxidation were as follows: RQ values, 0.94 to 1.12, and the ratios μmoles O_2 consumed per μmoles glucose oxidized, 1.65 to 2.02.

Effect of azide and 2,4-dinitrophenol on substrate oxidations. The oxygen uptake values for the oxidation of acetate, propionate, or glucose by suspensions of cells grown under different conditions (table 1) were all low when compared with the theoretical values for the complete oxidation of these substances to CO_2 and H_2O. Theoretical values for the oxidation of the substrates (moles O_2 required to completely oxidize a mole of substrate) and the observed oxygen uptakes (corrected for endogenous values) when the substances were completely removed by

cell suspensions of strain S were as follows: acetate, theoretical, 2.0, observed, 0.89 to 1.03; propionate, theoretical, 3.5, observed, 1.43 to 2.00; and glucose, theoretical, 6; observed, 1.65 to 2.02.

The low oxygen uptake values (as compared with the theoretical values) suggested that oxidative assimilation from the substrates was occurring during the oxidations by the cell suspensions. Both 2,4-dinitrophenol and sodium azide were tested as uncoupling agents for the oxidations, each over a range of concentrations. The oxygen uptake values for the substrate degradations were increased with suitable concentrations of each inhibitor; the most effective uncoupling concentrations for the quantity of cells used were for (a) 2,4-dinitrophenol, 0.00016 M and (b) sodium azide, 0.0033 M. When used at the effective uncoupling concentrations both substances decreased the rate of substrate oxidation (table 3).

TABLE 3

Effect of sodium azide and 2,4-dinitrophenol on the oxidation of substrates by cell suspensions

Inhibitor added	Rate of Oxidation (Q_{O_2}) for Cell Suspensions with:			
	No substrate	Acetate	Propionate	Glucose
Nil	16	34	27	29
Azide (0.0033 M)	24	14	3	17
2,4-Dinitrophenol (0.00016 M)	22	14	12	12
	Oxygen Uptakes (μmoles O_2 Consumed/μmole Substrate Removed) for Cells with:			
		Acetate	Propionate	Glucose
Nil		1.02	1.6	1.8
Azide (0.0033 M)		1.81	2.77	2.60
2,4-Dinitrophenol (0.00016 M)		1.52	2.40	2.10

The incubation systems contained cells (grown on a glucose-ammonium sulfate-thiamine medium for 48 hr) and phosphate buffer (0.066 M; pH 7.2). Additions to the systems: carbon substrates (10 μmoles/manometer cup) and inhibitors to give the final concentrations shown in the table. All respiration values for the substrates were corrected for the values obtained in the absence of the substrates.

Oxidative assimilation from C^{14}-labeled substrates. During the oxidation of substrates by cell suspensions of strain S, oxidative assimilation of part of the substrate was assumed to occur because of (a) the low over-all oxygen uptake figures for substrate oxidation and (b) the effect of uncoupling agents on the oxidations. Direct evidence for assimilation of carbon by cell suspensions from substrates was obtained from experiments in which C^{14}-labeled compounds (3-C^{14}-propionate, 1-C^{14}-acetate, and U-C^{14}-glucose) were incubated for a defined time with suspensions of strain S. The incubation system was subsequently divided into three fractions, namely, cells, supernatant, and carbon dioxide which was released by acidifying the whole system and trapping the gas in alkali. The fractions were assayed for C^{14} activity and the results (table 4) showed that C^{14} was assimilated from these substrates by strain S.

Release of assimilated carbon from cells in the presence and absence of substrates. Cell suspensions, which had been incubated with either 1-C^{14}-acetate, 3-C^{14}-propionate, or U-C^{14}-glucose, were washed and shaken aerobically with and without unlabeled substrates by the procedure described in Methods. The rates of release of $C^{14}O_2$ from the cells were determined and are

TABLE 4

Assimilation of C^{14} from labeled substrates by cell suspensions of strain S

	Substrates Oxidized by the Cell Suspensions		
	1-C^{14}-acetate	3-C^{14}-propionate	U-C^{14}-glucose
Cpm in the substrate added	20,950	17,650	37,950
Fractionation			
Cpm in the respired CO_2	6,490	7,250	10,380
Activity retained in cells (cpm)	11,200	6,315	24,825
Activity in the supernatant (cpm)	1,880	2,740	2,780
Total activity recovered (cpm)	19,570	16,305	37,985

Experimental details are given in Methods. The activities of the substrates added to incubation systems are given as the total number of cpm in 200 μmoles of each substance.

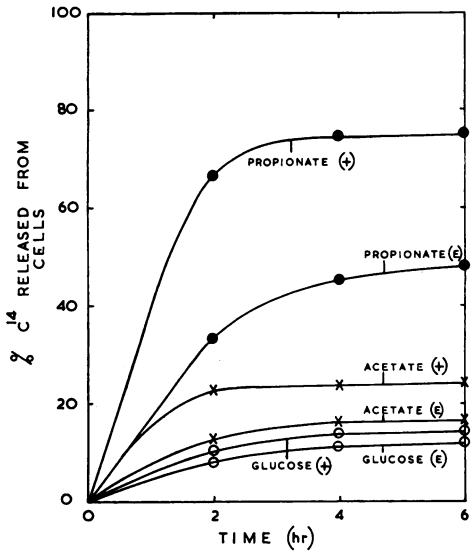

Figure 2. The release of assimilated C^{14} as $C^{14}O_2$ from cells (14 mg dry weight) which had been incubated with either 1-C^{14}-acetate, 3-C^{14}-propionate, or U-C^{14}-glucose and subsequently incubated alone (E) or with the same substrate (but unlabeled) used for the initial assimilation (+). Details of the experimental procedure are given in Methods.

TABLE 5

Distribution of assimilated C^{14} (from 3-C^{14}-propionate) in the cellular components of strain S

Sample Assayed	Total in Sample Assayed	Activity of the Original Cells (290 mg Dry Wt)
	cpm	%
Original cells	68,700	100
Ether-extractable lipid (83.5 mg)	25,400	36.8
Chloroform-extractable lipid (15.1 mg)		
Osazone	36,000	52.4

In each case, the sample, converted to CO_2 by wet combustion, was obtained from 290 mg dry weight of the cell suspension of strain S.

represented graphically in figure 2. Cells which had assimilated C^{14} from 1-C^{14}-acetate released (a) 18 per cent of the activity in 6 hr while respiring alone and (b) 25 per cent of the activity if the cells were oxidizing unlabeled sodium acetate during the same period. When U-C^{14}-glucose was the substrate from which assimilation occurred, a release of 13 per cent of the cellular activity (C^{14}) occurred in the absence of substrate in 5 hr and 16 per cent was released if the cells were simultaneously oxidizing unlabeled glucose. With cells which had assimilated C^{14} from 3-C^{14}-propionate, 48 per cent of the activity was released in 6 hr when the cells were shaken without added substrate while 71 per cent appeared in the same period if the cells were concurrently oxidizing unlabeled propionate.

Distribution of C^{14}, assimilated from substrates, in cell fractions. A preliminary fractionation of the cellular components, following the incubation of a suspension with 3-C^{14}-propionic acid, was carried out by the procedure described in Methods. Monosaccharide (glucose) obtained from the hydrolysis of cellular polysaccharide was converted to the osazone after the addition of carrier glucose. From the yield of osazone, after making an allowance for the carrier glucose used, it has been calculated that not more than 20 mg of monosaccharide (glucose) was released during the hydrolysis of 290 mg dry weight cells.

The activities of the fractions obtained from the separation of the cellular components are given in table 5.

DISCUSSION

Endogenous respiration. N. corallina strain S showed a high rate of endogenous respiration ($Q_{O_2}{}^E$) when prepared from growth media after 24 to 48 hr growth (table 1) and this rate was invariably lower for cells grown for longer times (72 to 96 hr). The low $Q_{O_2}{}^E$ values for the older cells were assumed to be due to the depletion of the reserves of cellular components which were the substrates for endogenous respiration.

The endogenous respiratory quotients for all of the cell suspensions studied (table 1) were within the narrow range of 0.89 to 1.00 even although (a) the cells had been grown on different carbon substrates and (b) the cells had been reaped from the growth media after different growth times. With cells grown on acetate as the carbon source and containing approximately 30 per cent lipid, the respiratory quotients ranged from 0.91 to 1.00 and in no instance approached the typical RQ values for cells oxidizing lipids. Accordingly, it has been considered likely that the immediate substrates for endogenous respiration are carbohydrates; the

inhibitions of endogenous respiration by sodium arsenite and sodium iodoacetate are consistent with this concept (table 2). The endogenous respiration rates of the various cell suspensions were increased with those concentrations of sodium azide and 2,4-dinitrophenol (table 2) which had been shown to be most effective in inhibiting oxidative assimilation during substrate oxidations. The increased $Q_{O_2}{}^E$ values with azide and 2,4-dinitrophenol are explicable if normal endogenous respiration is associated with some resynthesis of the endogenous substrate from an intermediate on the catabolic sequence, using energy from the oxidative degradations.

$$\text{Endogenous substrate} \underset{+\text{ energy}}{\overset{+\text{ O}}{\rightleftharpoons}}$$

$$\text{intermediate} \xrightarrow{+\text{ O}} CO_2 + H_2O$$

Cells respiring endogenously in the presence of the uncoupling agents would be degrading the endogenous substrates without concurrent resynthesis and could lead to the observed increased rates of endogenous oxygen uptake.

Oxidation of acetate by cell suspensions. The Q_{O_2} values for acetate oxidation by cells grown for 24 hr were approximately the same for each carbon nutrient used (table 1). However the $Q_{O_2}{}^{\text{acetate}}$ values were much lower for cells grown for longer periods and the reduction in these values for the older cells paralleled a reduction in the $Q_{O_2}{}^E$ values for the same cells. The observed low respiration values for older cells may be due to a decrease in the number of metabolically active cells in the older suspensions. However, on this basis cells having a zero rate of endogenous respiration should have zero metabolic activity against acetate. In fact, the cells retain a proportion of their initial metabolic activity which is independent of the extent of the endogenous respiration of the cells (figure 1; 72 and 96-hr-old cells).

The oxygen uptake curves for acetate oxidation by cells having no endogenous respiration showed an initial lag and a subsequent increase in the rate of oxidation (figure 1). The nature of the curves suggested that an autocatalytic process occurred during acetate oxidation by the cells. In young cells the oxidation of the endogenous substrates may be assumed to supply the citric acid cycle intermediates required for acetate oxidation (Santer and Ajl, 1954). However, strain S has been shown to possess isocitritase activity and, in cells having no endogenous respiration, the glyoxylate by-pass mechanism (Kornberg and Krebs, 1957) may be considered to supply citric acid cycle intermediates for the terminal oxidation of the acetate. Since the amounts of the cycle intermediates formed by this mechanism would increase with increasing rates of acetate oxidation (Wong and Ajl, 1957), the entire process for acetate oxidation could show the characteristics of an autocatalytic system. If the inflected acetate oxidation curves (figure 1) were, in fact, due to a limited supply of citric acid cycle intermediates, the inflexion should be removed if a substance such as succinate was added to the cells showing no endogenous respiration. In a typical experiment, cells (96-hr-old) oxidizing acetate were compared with cells oxidizing a small amount of succinate (2 μmoles) in the presence of acetate (10 μmoles). The presence of succinate considerably shortened the lag on the acetate oxygen uptake curve (after corrections had been made for succinate oxidation). Cell suspensions which retain metabolic activity when all endogenous respiration has ceased, constitute a less complicated system and may prove to be more useful for studying whole cell metabolism than the conventional resting cell suspensions.

Oxidative assimilation was shown to have occurred during the oxidation of acetate by cell suspensions of strain S from the following results: (a) the oxygen uptake values for acetate removal by the organism were equivalent to approximately 50 per cent of the theoretical values for complete oxidation (table 1); (b) in the presence of suitable concentrations of sodium azide and 2,4-dinitrophenol the oxygen uptakes for acetate oxidation could be increased (table 3); (c) cell suspensions oxidizing 1-C^{14}-acetate incorporated 57 per cent of the C^{14} into the cells (table 4). The ability of the cells to assimilate part of the acetate oxidized was independent of (a) the age and (b) the $Q_{O_2}{}^E$ values of the cells (table 1). The processes for acetate oxidation (catabolism) and oxidative assimilation from acetate (anabolism) appear to be geared together.

Oxidation of propionate and glucose by cell suspensions. The Q_{O_2} values for propionate and glucose oxidations decreased as cells of increasing

age were used in the incubation systems and the relationship between $Q_{O_2}{}^{substrate}$ and $Q_{O_2}{}^{E}$ for the cells was the same as had been observed for acetate oxidation.

Oxidative assimilation occurred during the oxidations of propionate and glucose (table 3); the extent of the assimilation was independent of (a) the growth time for cells used and (b) the $Q_{O_2}{}^{E}$ values of the cells. The assimilatory and oxidative mechanisms for these substrates (as for acetate) appeared to be closely interrelated.

Inhibition of oxidative assimilation. The total oxygen uptake values for the complete removal of acetate, propionate and glucose by cell suspensions of strain S were all increased in the presence of certain concentrations of sodium azide or 2,4-dinitrophenol (table 3). In no instance however, did the oxygen uptake in the presence of the inhibitor reach the theoretical value for complete oxidation of the substrate, even although isotope experiments (table 4) had indicated that the low oxygen uptakes were due to the assimilation of all of the substrate not oxidized. With reference to acetate oxidation by cell suspensions, the oxygen uptake for the complete removal of the substrate was equal to approximately 50 per cent of the theoretical value for complete oxidation (table 1) and the amount of C^{14} assimilated from 1-C^{14}-acetate was equal to approximately 50 per cent of the total activity of the acetate added to the cells (table 4).

Although the inhibition of oxidative assimilation leads to an increased over-all oxygen uptake for a substrate, the rate of oxidation of the substrate is usually reduced by the inhibitor. With concentrations of inhibitor capable of completely uncoupling oxidative assimilation (and so giving the theoretical oxygen uptake for complete oxidation of the substrate) the rate of oxidation is usually depressed to such an extent that the time required for the oxidation to go to completion is impractically prolonged. The experimental results suggest that the enzymes for substrate oxidation are more readily inhibited by the uncoupling agents than the mechanisms for assimilation from substrates.

Assimilation of C^{14} from labeled substrates and the subsequent release of C^{14} from the cells. Direct evidence was obtained for the occurrence or oxidative assimilation in strain S from the observations that during the oxidation of C^{14}-labeled substrates, the isotope was incorporated into the cellular constituents (table 4). The extent of the assimilation estimated from the percentage of the substrate C^{14} incorporated into the cells, was approximately the same as the estimates made from the low over-all oxygen uptake figures for the oxidation of the substrates by cell suspensions.

The rates of release of C^{14} from cells which had been preincubated with labeled substrates showed that, if the cells were concurrently oxidizing an unlabeled substrate, the rate of release of C^{14} may be different from the values for the cells respiring endogenously (figure 2). Accordingly, no general rule may be made concerning the validity of correcting the respiration values for substrate oxidation by whole cells for the endogenous respiration figures. The presence or absence of glucose did not markedly change the rate of release of C^{14} from cells which had assimilated carbon from this substrate. However, with cells which had previously oxidized 3-C^{14}-propionate, the release of C^{14} was considerably increased in the presence of unlabeled propionate by comparison with the same cells respiring endogenously. The incorporation of C^{14} from propionate appears to lead to the synthesis of a labile cellular constituent which is more rapidly metabolized in the presence of unlabeled propionate than during cellular respiration in the absence of added substrates. Similar results have been observed with other microorganisms (Gibbs and Wood, 1952). When *Pseudomonas fluorescens* was incubated with C^{14}-labeled glucose for a short period, the cells showed an increased release of $C^{14}O_2$ if unlabeled glucose was added to the cells, by comparison with the cells respiring endogenously. Cells grown on U-C^{14}-glucose, showed the same rate of release of $C^{14}O_2$ irrespective of whether or not the cells were oxidizing C^{12}-glucose (Gibbs and Wood, 1952).

The preliminary experiments carried out to determine the distribution of C^{14} in cells which had oxidatively assimilated the isotope from labeled substrates (table 5) showed that although 37 per cent of the counts were recovered in the lipid fraction (equivalent to approximately 30 per cent of the dry cell weight), 52 per cent were present in a carbohydrate fraction which constituted only a very small percentage of cellular material (less than 8 per cent). The very high activity of the carbohydrate fraction suggested

that carbohydrate may be the immediate cellular constituent on the assimilation pathway.

SUMMARY

The respiratory quotients for the endogenous respiration of *Nocardia corallina* strain S, grown for different times on various carbon substrates, were all in the range 0.89 to 1.00. The rates of endogenous respiration were increased with certain concentrations of sodium azide and 2,4-dinitrophenol. The relationship between (a) the rate of oxidation of acetate, propionate, and glucose and (b) the rate of endogenous respiration of the cells used, was examined. Cells showing no endogenous respiration oxidized acetate. Oxidative assimilation was shown to occur during the oxidation of 1-C^{14}-acetate, 3-C^{14}-propionate, and U-C^{14}-glucose by cell suspensions. The release of C^{14} from the labeled cells was faster for cells oxidizing unlabeled propionate and acetate than for cells respiring endogenously. Glucose had little affect on the release of $C^{14}O_2$ from the cells. The distribution of C^{14} in the constituents of cells which had oxidized C^{14}-labeled substrates was studied.

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