NITROGENOUS COMPOUNDS AS SUBSTRATES FOR ENDOGENOUS RESPIRATION IN MICROORGANISMS[1]

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The endogenous substrate of *Pseudomonas aeruginosa* has been reported to be nitrogenous in nature, as was evident by the marked and quantitatively predictable production of ammonia during endogenous respiration and it was concluded that amino acids were the nitrogenous substrates involved. Further, it was shown that on the addition of an oxidizable substrate, the ammonia which had accumulated in the medium was rapidly assimilated by the resting cells (Warren, Ells, and Campbell, 1960). Although Lamanna and Mallette (1953) state that endogenous substrates vary with the different bacterial species, little definite information as to their actual nature is available. It was felt that an indication of the general distribution and importance of nitrogenous endogenous substrates could be established by correlating ammonia production with the endogenous oxygen consumption of a number of bacterial species. In addition, the general significance of the observation that endogenously produced ammonia was reincorporated into the cell on the addition of oxidizable substrate, could be determined.

MATERIALS AND METHODS

P. aeruginosa, *Pseudomonas fluorescens*, *Achromobacter* sp., and *Aerobacter aerogenes* were grown in a glucose, ammonium phosphate-salts medium. *Escherichia coli*, *Saccharomyces cerevisiae*, and *Bacillus subtilis* were grown in a medium consisting of 1% tryptone (Difco), 0.5% yeast extract, 1.0% glucose, and 0.5% K_2HPO_4. A supplement of 0.01% thiamine was added to enhance the growth of *B. subtilis*. The cells were harvested after 20 hr incubation at 30 C. *Streptococcus faecalis* was grown in a medium composed of 1.0% tryptic digest of casein, 0.5% yeast extract, 0.25% K_2HPO_4, and 1.0% glucose. This organism was harvested after 17 hr incubation at 30 C. Ammonia was determined by the Conway microdiffusion technique (Conway, 1950) and dry weights were established by drying duplicate 5-ml suspensions of cells to constant weight at 100 C. Oxygen consumption was measured in the conventional Warburg apparatus at 30 C. Some of the organisms studied displayed low rates of endogenous respiration thus necessitating the use of very concentrated suspensions of cells to ensure that the level of ammonia production would be sufficiently high for accurate measurement. The final concentration of resting cells in the Warburg vessels was 3.7 × growth concentration for the pseudomonads, *A. aerogenes*, *E. coli*, *S. cerevisiae*, and *B. subtilis*; 8.5 × growth concentration for the *Achromobacter* species; and 40 × growth concentration for the *Streptococcus* species.

The zero time values for ammonia were obtained by analysis of the contents of a duplicate Warburg vessel removed at the time of tipping glucose into those vessels receiving glucose at zero time.

RESULTS AND DISCUSSION

With the possible exception of *A. aerogenes*, all of the organisms examined apparently utilized nitrogenous substrates during endogenous respiration as was evident by the production of ammonia (Table 1). Moreover, with the exception of *S. faecalis*, the organisms reincorporated the ammonia after the addition of glucose. *B. subtilis* was relatively slow in this reincorporation but this may be just a manifestation of the relatively slow metabolic rate of the organism. An explanation of these data is that the disappearance of ammonia on the addition of glucose is the basic process of oxidative assimilation. The observation that *S. faecalis* did not reincorporate ammonia agrees with the fact that this organism has only a limited ability to utilize inorganic ammonium salts for synthesis of cellular material.

If the substrate of endogenous respiration was protein, then a reasonable ratio of oxygen/ammonia for the complete oxidation of the substrate

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TABLE 1

Ammonia production during endogenous respiration and reincorporation on addition of glucose

Microorganism	Ammonia Production (μg/100 mg Dry Wt Cells)								
	Endogenous			Glucose added at 1 hr			Glucose added at zero time		
	0 hr	1 hr	2 hr	0 hr	1 hr	2 hr	0 hr	1 hr	2 hr
Pseudomonas aeruginosa ATCC 9027	103	253	447	103	253	0	103	0	0
Pseudomonas aeruginosa 120Na	187	292	387	187	292	0	187	0	0
Pseudomonas fluorescens A.3.12	229	410	586	229	410	0	229	0	0
Achromobacter sp.	99	149	220	99	149	0	99	0	0
Escherichia coli ATCC 6894	59	118	194	59	118	0	59	0	0
Aerobacter aerogenes	0	0	0	0	0	0	0	0	0
Bacillus subtilis ATCC 6633	293	377	444	293	377	277	293	193	0
Saccharomyces cerevisiae	99	216	278	99	216	51	99	0	0
Streptococcus faecalis ATCC 8043	59	92	112	59	92	96	59	92	118

would be 4.5 which is the value for glutamic acid, an average amino acid in this respect. The values for the three strains of pseudomonads are very close to this (Table 2). A higher value, such as that obtained for *E. coli*, would indicate that an almost equal amount of carbohydrate or fat also served as endogenous substrate. The infinitely high value obtained for *A. aerogenes* need not mean that ammonia was not produced, but that the polysaccharide reserves of the organism were such that the ammonia was assimilated as rapidly as it was produced, as was the case when glucose was added to suspensions of the other organisms. The lower values, such as those obtained for *S. faecalis* and *B. subtilis*, indicate either that the amino acids were not oxidized to completion or that compounds such as glycine and alanine, which require smaller amounts of oxygen for complete oxidation, formed a major part of the substrate. The ratio for *S. faecalis* differed markedly from experiment to experiment perhaps indicating that, depending on undefined growth conditions, the organism had different endogenous reserves to draw on or that it oxidized its endogenous reserves to varying degrees of completeness.

The data obtained do not disagree with the information in the literature but they do give a more quantitative assessment of the nitrogenous reserves.

If a glycogen-like compound was the reserve material in *P. aeruginosa*, the pathway of endogenous respiration would involve derivatives of glucose and it is probable that the organism would exhibit a constitutive enzyme system for the degradation of glucose. However, the organism under study does not have a constitutive system for glucose utilization (Ney, 1948) and, therefore, probably does not have a glycogen-like compound as a reserve material. Moreover, Forsyth, Hayward, and Roberts (1958) were not able to demonstrate the presence of poly-β-hydroxybutyric acid in the green pigmented pseudomonads. Thus, the conclusion that a proteinaceous material is the endogenous substrate is in order. *A. aerogenes* has been shown to store intracellular polysaccharide (Duguid and Wilkinson, 1953) and, as it is unable to utilize its own extracellular polysaccharide, it has been suggested that its rapid rate of endogenous respiration is due to the metabolism of the stored intracellular polysaccharide (Wilkinson, 1958). Although *E. coli* has been shown to store both lipid and polysaccharide (Dagley and Johnson, 1953), it has also been shown to possess internal nitrogen reserves (Roberts et al., 1955; Mandelstam, 1958) and it appears that it draws on several of these reserve materials during endogenous respiration. Glycogen and trehalose are reported to be the principal reserve carbohydrates of *S. cerevisiae* (Lindegren, 1945; Stewart, Richtmyer, and Hudson, 1950). However, this organism also possesses an internal amino acid pool (Halvorson and Spiegelman, 1952) which probably serves as a major, initial endogenous substrate.

The reserve material which is utilized by the pseudomonads during the 2-hr period of aeration

TABLE 2

Endogenous oxygen uptake as a function of ammonia production

Microorganism	O_2 Uptake	NH_3 Evolved	O_2/NH_3
	μmoles: 2 hr: 100 mg dry wt cells		
Pseudomonas aeruginosa ATCC 9027	88	20.2	4.34
Pseudomonas aeruginosa 120Na	55.5	11.8	4.70
Pseudomonas fluorescens A.3.12	98.0	21.0	4.65
Achromobacter sp.	45.1	7.14	6.33
Escherichia coli ATCC 6894	79.0	7.94	9.95
Aerobacter aerogenes	69.3	0	∞
Bacillus subtilis ATCC 6633	29.8	8.9	3.36
Saccharomyces cerevisiae	57.0	10.5	5.43
Streptococcus faecalis ATCC 8043	8.0	3.15	2.54

would appear to be protein, for the concentration of free amino acids has been shown to increase during this period (Warren et al., 1960). However, this need not be true for the other organisms. In particular, the amount of ammonia released by *S. faecalis* could be accounted for by the oxidation of free amino acids. The data in the present study do not permit the determination of the relative importance of protein and free amino acids as sources of endogenous ammonia. From their studies with *Sarcina lutea*, Dawes and Holms (1958) concluded that the internal amino acid pool was the sole endogenous substrate and, when this pool was decreased to approximately one-half, the endogenous metabolism fell to a negligible level.

SUMMARY

Nine microorganisms were tested for their ability to produce ammonia during endogenous respiration. *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Achromobacter* species, *Escherichia coli*, *Bacillus subtilis*, *Saccharomyces cerevisiae*, and *Streptococcus faecalis* consistently produced appreciable quantities of ammonia. The strain of *Aerobacter aerogenes* used produced no detectable quantities of ammonia. When glucose was added to respiring cell suspensions, all organisms except *S. faecalis* reincorporated the accumulated ammonia. When the ratio of oxygen consumed to ammonia evolved was determined, it was found that the pseudomonads consistently had values of 4.5, indicating that the endogenous substrates could have been proteins which had been oxidized to completion. The ratios for *B. subtilis* and *S. faecilis* were lower, indicating that the endogenous substrates contained a high percentage of nitrogen or that the substrates were incompletely oxidized. The other organisms gave values greater than 4.5, indicating that nonnitrogenous compounds were also serving as endogenous substrates for these organisms.

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