ENDOGENOUS RESPIRATION OF *STAPHYLOCOCCUS AUREUS*

H. H. RAMSEY

Department of Medical Microbiology, Stanford University, Palo Alto, California, and Research Corporation, Burlingame, California

Received for publication September 5, 1961

ABSTRACT

RAMSEY, H. H. (Stanford University, Palo Alto, Calif.). Endogenous respiration of *Staphylococcus aureus*. J. Bacteriol. **83**:507–514. 1962.—The endogenous respiration of *Staphylococcus aureus* is dependent upon the medium used to grow the cell suspension. Within wide ranges, the concentration of glucose in the medium has no effect upon subsequent endogenous respiration of the cells, but the concentration of amino acids in the medium, within certain limits, has a very marked effect. The total carbohydrate content of the cells does not decrease during endogenous respiration. As endogenous respiration proceeds, ammonia appears in the supernatant, and the concentration of glutamic acid in the free amino acid pool decreases. Organisms grown in the presence of labeled glutamic acid liberate labeled CO_2 when allowed to respire without added substrate. The principal source of this CO_2 is the free glutamate in the metabolic pool; its liberation is not suppressed by exogenous glucose or glutamate. With totally labeled cells, the free pool undergoes a rapid, but not total, depletion and remains at a low level for a long time. Activity of the protein fraction declines with time and shows the largest net decrease of all fractions. Exogenous glucose does not inhibit the release of labeled CO_2 by totally labeled cells. Other amino acids in the free pool which can serve as endogenous substrates are aspartic acid and, to much lesser extents, glycine and alanine. The results indicate that both free amino acids and cellular protein may serve as endogenous substrates of *S. aureus*.

The problem of endogenous respiration has been a source of difficulty for workers in microbial physiology since the inception of manometric techniques. In calculating manometric data, it is necessary to know what effect, if any, the addition of exogenous substrate has upon endogenous respiration. It has been rather conclusively demonstrated that the effect is dependent upon the organism under investigation, the condition under which it is grown, and the exogenous substrate being used. Barker (1936), studying *Prototheca zopfii*, was the first to suggest that easily oxidizable substrates tend to suppress endogenous respiration while slowly oxidized substances do not. Similar conclusions have been reached by Doudoroff (1940) with *Pseudomonas saccharophila* and by van Niel and Cohen (1942) with *Candida albicans*. Wiame and Doudoroff (1951) used labeled substrates and concluded that 96% of the endogenous respiration of *P. saccharophila* was inhibited by the addition of exogenous glucose. Cochrane and Gibbs (1951) used similar techniques but a different organism (*Streptomyces coelicolor*), and concluded that exogenous substrates exerted no depressive action on endogenous respiration. Norris, Campbell, and Ney (1949), working with *P. aeruginosa*, were also unable to obtain evidence that exogenous substrate suppressed endogenous respiration, and also noted that failure to correct for endogenous respiration resulted in oxygen uptake in excess of that required for complete oxidation of the exogenous substrate. Similar results have been noted for *Bacillus subtilis* (Wilner and Clifton, 1954) and for *Hydrogenomonas facilis* (Marino and Clifton, 1955). A case of apparent stimulation of endogenous respiration by exogenous substrate has been noted by Reiner, Gest, and Kamen (1949) with *Saccharomyces cerevisiae*.

Blumenthal, Koffler, and Heath (1957), working with *Penicillium chrysogenum*, demonstrated the effect of previous growth conditions on the subsequent interrelationship between exogenous and endogenous respiration. When the organism was grown on C^{14}-glucose, the rate of release of $C^{14}O_2$ by resting cells was similar under endogenous conditions and when acetate or glucose was present, indicating no suppression of endogenous

respiration. The endogenous respiration of cells grown on C^{14}-acetate was inhibited by acetate but not by glucose.

The usual objective in manometric experiments is to study the ability of an organism to oxidize a certain exogenous compound. For this reason, endogenous respiration is considered to be an interfering factor, and efforts are made either to grow cells with a low endogenous activity (Wood and Gunsalus, 1942) or to reduce it to a negligible value by "starving" cells in a nonnutrient medium prior to use (Quastel and Whetham, 1924).

Relatively few studies have been made on the nature of endogenous respiration. Perhaps the first detailed study was that of Stier and Stannard (1936*a*), who noted that the rate of endogenous respiration of yeast declined with time. The decline was found to be characteristic of a first-order process, was not due to enzyme deterioration, and evidence was obtained that the endogenous substrate was carbohydrate. Subsequently (Stier and Stannard, 1936*b*), the dissimilation of intracellular reserves was shown to be respiratory in nature, and the suggestion was made that the term endogenous respiration be used instead of the older term autofermentation. More recent work with yeast (Eaton, 1960) has demonstrated at least three sources of endogenous respiration in this organism. One source is trehalose, which is utilized only anaerobically. The other sources consist of two distinct glycogen pools; one is utilized either aerobically or anaerobically and the other is utilized only anaerobically, along with trehalose.

Dawes and Holms (1958) and Warren, Ells, and Campbell (1960), working with *Sarcina lutea* and *P. aeruginosa*, respectively, found that nitrogenous compounds served as endogenous substrates. Also, Bardi and Boretti (1958) demonstrated the importance of free amino acids as endogenous substrates for *Nocardia rugosa*. Oxidation of these amino acids, and of the proteins from which they were derived, was inhibited by exogenous glucose. Gronlund and Campbell (1961) implicated nitrogenous compounds as endogenous substrates in a variety of organisms, including *P. fluorescens*, *Achromobacter* spp., *Escherichia coli*, *B. subtilis*, *S. cerevisiae*, and *Streptococcus faecalis*.

The investigation described herein is concerned with the endogenous respiration of *S. aureus*. The effect of growth conditions and other variables on endogenous respiration has been studied. It is suggested that free amino acids and cellular protein can serve as substrates for endogenous respiration. No evidence has been obtained which would suggest that intracellular carbohydrate serves as a source of endogenous metabolism.

MATERIALS AND METHODS

The test organism was a strain of *S. aureus* which has been described previously (Ramsey and Padron, 1954). Stock cultures were maintained on slopes of A C agar (Bacto A C broth supplemented with 2% agar, Difco Laboratories, Detroit, Mich.). Cell suspensions for manometry were obtained by inoculating Roux bottles containing 100 ml of the desired medium with 2 ml of a washed, 24-hr culture grown in a defined medium (Ramsey and Padron, 1954). Unless otherwise noted, the cells were harvested at 21 hr in 0.05 M phosphate buffer (pH 7.2), washed, and resuspended to a final volume of 6 ml per Roux bottle. A 1-ml quantity of this suspension was added to the main compartment of each Warburg vessel; the total volume in each flask was 2.4 ml. Conventional manometric techniques were used throughout at 37 C, and dry weights were taken for calculations of respiration.

To obtain radioactive cells, the organisms were grown in broth containing labeled glucose, glutamic acid, or algal hydrolyzate, both with and without added unlabeled carrier. In some instances, unlabeled cells were incubated for 1 hr at 37 C in the salts mixture of Gale (1947) with added labeled amino acid. Radioactive carbon dioxide was determined as $C^{14}O_2$ on a Dynacon electrometer (Nuclear-Chicago) after release from KOH with acid. The activity of dried samples was determined by complete combustion and collection of CO_2 in a combustion train.

To determine the degree of labeling of each cellular fraction, the cells were treated as described by Park and Hancock (1960), except for elimination of trypsin digestion. Cells thus treated were separated into four fractions: a cold trichloroacetic acid (TCA) fraction containing free amino acids and other low molecular weight compounds, an aqueous ethanol fraction containing lipid and "protein", a hot TCA fraction containing nucleic acids and teichoic acid, and a residue containing 95% of the cell protein and the mucopeptide of the cell wall.

Ammonia was determined according to the method described by Umbreit, Burris, and Stauffer (1957). Total carbohydrate was assayed according to Shetlar, Foster, and Everett (1948). Free glutamic acid was measured, using the decarboxylase method of Gale (1947), with *E. coli* strain K-12 as the test organism.

RESULTS

The effect of age of cells on endogenous respiration was determined by growing organisms for 12, 24, 48, and 72 hr on A C agar. The endogenous Q_{O_2} values for the cells were found to be 14.8, 12.0, 10.0, and 0, respectively. However, the ability to oxidize exogenous pyruvate remained unchanged over the age intervals studied, indicating that the difference in endogenous activity was due to the exhaustion of intracellular substrate rather than to a loss of enzyme or coenzyme activity. These results are similar to those obtained with *N. corallina* by Midwinter and Batt (1960), who observed that endogenous respiration decreased with the age of the culture. Midwinter and Batt obtained respiratory quotient (RQ) values ranging from 0.89 to 1.00, and these values were independent of the age of the cells and the substrate on which they were grown. In the present study, the RQ values ranged from 0.83 to 0.86.

Organisms grown on A C agar were compared with organisms grown on a defined medium containing 0.2% Casamino acids (Difco), 2% glucose, vitamins, salts, 0.005% cystine and tryptophan, and 1.5% agar. It was found that organisms harvested from the complex medium had an endogenous Q_{O_2} value of 18.2, compared to a value of 9.6 for organisms grown on the defined medium. Also, the cell yield, in terms of dry weight, was much higher from the complex medium (108.6 mg per Roux bottle) than from the defined medium (12.6 mg per Roux bottle).

To determine which constituent of the complex medium was responsible for the increased endogenous respiration, the individual constituents were tested for activity when added as supplements to the defined medium. Yeast extract, malt extract, and beef extract (all Difco) were added at final concentrations of 0.3% and Proteose Peptone No. 3 (Difco) at a final concentration of 2.0% (concentrations equal to those found in the A C agar). Also, a combination of yeast extract and Proteose Peptone No. 3 was tested for activity. It was found that malt extract was inhibitory to growth and resulted in a lower Q_{O_2} value; beef extract was without effect (results not included). However, both yeast extract and Proteose Peptone No. 3 were quite stimulatory to growth and resulted in an increase in the endogenous Q_{O_2} values (Table 1); the yeast extract produced cells with the higher endogenous Q_{O_2} value. A combination of the two was not additive and was actually inferior to yeast extract alone. These results would indicate that complex materials which result in greater cell yields are also responsible for storage of endogenous reserve substrates. Whether or not the supplements act directly in increasing the cell mass and endogenous respiration is not known. The effect may be indirect in that the supplements could provide stimulatory factors which enable the organism to assimilate more efficiently compounds present in the defined medium.

TABLE 1. *Effect of adding supplements to the defined medium on resultant dry weight and endogenous respiration of Staphylococcus aureus*

Supplement	Dry weight of cells		Q_{O_2}	Increase Q_{O_2}, %
	mg/100 ml of medium	Increase, %		
None	26.4		9.3	
Yeast extract, 0.3%	76.2	189	15.7	42
Proteose Peptone No. 3, 2.0%	144.6	448	11.6	20
Proteose Peptone No. 3, 2.0%, plus yeast extract, 0.3%	136.8	420	13.6	33

Although this question was not tested critically, it is pertinent to note that stimulation of growth was much greater than stimulation of endogenous respiration. For example, yeast extract increased the cell yield by 189% but increased endogenous Q_{O_2} by only 42%. Further, when yeast extract was added to cells actively respiring in a Warburg vessel, it failed to enhance the rate of endogenous respiration. It would appear, therefore, that stimulation of endogenous respiration by yeast extract is closely associated with the phenomena of growth and cell division.

In an attempt to obtain cells with an endogenous respiration comparable with that of cells

grown on agar, organisms were grown on the defined medium containing varying concentrations of glucose and amino acids. It was observed that, with glucose concentrations ranging from 0.5 to 7.5%, the dry weight varied only from 19.6 to 26.4 mg per Roux bottle and the endogenous Q_{O_2} varied only from 8.4 to 11.8. An increase of the glucose to 10% produced a smaller cell crop and a decreased endogenous respiration.

A similar experiment was performed in which the Casamino acid concentration was varied from 0.025 to 1.0%. With concentrations between 0.1 to 1.0%, the cell yield was roughly proportionate to the concentration of Casamino acids, but the endogenous Q_{O_2} values fell within a narrow range of 10.0 to 11.4. With concentrations of 0.05 and 0.025%, the Q_{O_2} decreased to 6.5 and 4.8, respectively. It should be noted, however, that in numerous replicate experiments wide discrepancies have been found with cells grown on the lower concentrations. In some experiments, no respiration could be detected; in others respiration was abnormally high. Perhaps one explanation of this variation was the low yield of cells obtained with the lower concentrations of amino acids. The growth on 0.025% Casamino acids averaged only 10.2 mg per Roux bottle, and that on 0.05% Casamino acids averaged only 15.0 mg per Roux bottle. With such a low cell yield, any variation or experimental error will be magnified. Further, cells grown on low nitrogen medium were quite granular and difficult to pipet. The probability is quite good that variations occurred in the amount of cells introduced into replicate vessels and into the weighing pans. These difficulties were not encountered with cells grown on concentrations of Casamino acids of 0.1% or higher.

TABLE 2. *Ammonia liberation and oxygen consumption of cells respiring in the presence and absence of exogenous glucose*

Time	Endogenous		Glucose*	
	O_2 uptake	NH_3 liberated	O_2 uptake	NH_3 liberated
min	*μliters*	*μg/ml*	*μliters*	*μg/ml*
0	0	8†	0	8†
30	113	20.5	245	13.0
60	173	22.0	332	10.0
90	215	26.0	385	17.0
135	283		430	20.3
165	310		458	24.8

* A 0.1-ml quantity of 0.1 M glucose tipped into main compartment at zero time.

† This value for NH_3 liberation represents that amount of ammonia liberated while the cells were being resuspended, introduced into the flasks, and equilibrated for 3 min.

TABLE 3. *Liberation of labeled CO_2 (in mμc) by cells grown in AC broth supplemented with U-C^{14}-glutamic acid**

Time	Substrate added		
	None	Glucose, 0.1 ml, 0.1 M	Glutamate, 0.1 ml, 0.1 M
min			
0	21.27		
30	39.76	43.65	41.49
60	45.38	57.92	48.41
150†	52.30	57.49	60.08

* Per cent recovery of total radioactivity added to medium: 91.

† O_2 (μliters) consumed in 150 min: endogenous, 113; glucose, 1114; glutamate, 966.

The foregoing results demonstrate the effect of previous cultural conditions on subsequent endogenous respiration. However, they do not provide evidence on the nature of the endogenous substrate itself. That the substrate is not carbohydrate in nature was shown by the following experiment. Cells were grown on defined medium and introduced into Warburg flasks; at 0, 30, 60, and 90 min, samples were taken, washed, and assayed for total carbohydrate. The carbohydrate content of the cells at harvest was found to be 11.8 μg per mg, and there was no change during the course of the experiment. Similar results were obtained with cells grown on the defined medium supplemented with yeast extract.

Cells respiring under endogenous conditions liberate ammonia (Table 2). For this experiment, cells were grown on the defined medium supplemented with 0.1% yeast extract; the cells were then placed in the vessels, and the supernatant fluid examined for ammonia at various time intervals. Ammonia was liberated in increasing concentrations as endogenous respiration proceeded. The total amount of ammonia liberated was 26 μg (1.53 μmoles), and the amount of oxygen consumed during the same time interval was 215 μliters (9.5 μmoles). If one were to assume that the endogenous substrate(s) was an amino acid, or a group of amino acids (Warren

et al., 1960), the theoretical oxygen uptake for complete oxidation, on the basis of ammonia liberated, would be 154.2 μliters or 70% of that observed. The discrepancy is probably explained by the fact that the values do not include free intracellular ammonia (Warren et al., 1960), nor do they account for possible reassimilation of ammonia. When the free amino acid pools of these same cells were extracted according to the method of Gale (1947), chromatographed in butanol–acetic acid–H_2O (4:1:1), and sprayed with ninhydrin, a group of amino acids was observed. One of these, corresponding in R_F to glutamic acid (Block, 1952), decreased in intensity until, at 90 min, it was only faintly detectable. This spot could be replaced by adding a known concentration of glutamic acid to the free amino acid pool prior to spotting on the paper.

In a second series of flasks, the effect of exogenous substrate on endogenous metabolism was measured by tipping 0.1 ml of 0.1 M glucose into the main compartment at zero time and comparing the ammonia liberated with that of cells respiring without added substrate (Table 2). The introduction of glucose decreased the amount of ammonia liberated. However, the inhibition was not complete. Cells in a third series of flasks were allowed to respire for periods of 75 and 105 min after the exhaustion of glucose, and the supernatants were collected for ammonia analysis. Analysis demonstrated ammonia liberation of 20.3 and 24.8 μg/ml, values comparable with that obtained in the endogenous control flasks. These results would suggest that exogenous glucose only partially inhibits the oxidation of intracellular nitrogenous substrates. Once the extracellular glucose is exhausted, oxidation of endogenous nitrogenous substrates continues until the ammonia liberated approaches that of cell suspensions which have received no exogenous glucose.

Cells grown in broth supplemented with C^{14}-glutamate liberate radioactive carbon dioxide (Table 3). Of a total cellular activity of 133.7 mμc, 52.3 mμc (39.2%) were liberated as CO_2 by the cells respiring without added substrate. Cells oxidizing unlabeled glucose or glutamate liberated slightly larger amounts of labeled CO_2. In a parallel experiment, the location of intracellular label and its disposition during endogenous respiration were determined by the fractionation procedures of Park and Hancock (1960). The highest activity was found in the fraction containing protein and cell wall (Table 4). However, the free metabolic pool (soluble in 5% TCA) underwent the greatest decrease (44.92 mμc or 85.3%) during 150 min of endogenous respiration. Part of this decrease (3.34 mμc) may be accounted for by an increase in activity of the ethanol-soluble fraction. However, of the net loss in radioactivity of all fractions (56.42 mμc), 44.92 mμc, or 77%, is attributable to the free-pool fraction containing low molecular weight compounds. Chromatography of the free pool revealed a single radioactive area corresponding in R_F to glutamic acid.

The above results suggest that free glutamate can serve as an endogenous substrate in *S. aureus*. To determine whether or not the glutamate content of the free pool could account for the entire endogenous respiration, a Warburg experiment was performed and, concurrently, quantitative assays were made of glutamic acid in the free amino acid pool. With a dry weight of 36.6 mg, the cells contained 0.5 μmole of free glutamate at zero time; the glutamate content at 48 min

TABLE 4. *Distribution of C^{14} (mμc) in cell fractions of Staphylococcus aureus grown in AC broth supplemented with U-C^{14}-glutamic acid**

Time	Cold TCA extract	Hot TCA extract	Ethanol-soluble lipid and "protein"	Residual protein and cell wall
min				
0	52.67	5.88	No activity	137.70
60	10.43	4.14	7.75	128.34
150	7.75	4.41	3.34	124.33
Change	−44.92	−1.47	+3.34	−13.37
Change, %	−85.29	−25.00		−9.71

* Total activity of cells: 240.64 mμc. Activity of carbon dioxide at 150 min: 47.75 mμc. Per cent recovery of total radioactivity in cells: 96.

TABLE 5. *Distribution of C^{14} (mμc) in cell fractions, carbon dioxide, and supernatant of uniformly labeled Staphylococcus aureus**

Time	Cold TCA extract	Hot TCA extract	Ethanol-soluble lipid and protein	Residual protein and cell wall	CO_2	Supernatant
min						
0	5.5	6.8	4.8	39.2		
30	3.8	16.1	3.0	28.0	1.2	1.9
80	3.2	13.4	3.6	30.8	2.5	2.5
360	3.1	15.2	3.4	23.1	6.6	5.7
Net change	−2.4	+8.4	−1.4	−16.1	+6.6	+5.7

* Per cent recovery of total radioactivity of cells: 84 to 88.

declined to 0.35 μmole, and at 96 min to 0.1 μmole. Corresponding oxygen consumption values were 443 μliters at 48 min and 710 μliters at 96 min. By comparing the amount of oxygen consumed with the amount of free glutamate available, it becomes obvious that glutamate is not the sole endogenous substrate. Assuming a value of 4.5 μmoles O_2 consumed per μmole glutamate, the amount of free glutamate available could account for only 2.25 μmoles or 50.4 μliters of oxygen.

When uniformly labeled cells (obtained by growing the organism in A C broth supplemented with U-C^{14}-glucose and C^{14}-algal hydrolyzate) were fractionated, a different pattern of distribution and disposition of label was observed (Table 5) from that obtained with glutamate-labeled cells. The cold TCA-soluble fraction and the ethanol-soluble fraction both diminished during the first 30 min of endogenous respiration, and then tended to remain constant over the next 330 min. The residue portion showed a net decrease over the entire interval of observation, whereas the hot-TCA fraction more than doubled during the first 30 min and maintained this high degree of activity. It thus appears that the free pool rapidly undergoes partial depletion, but, in the time interval studied, does not become completely exhausted. It is possible that the pool, upon reaching a certain level, is replenished through degradation of cellular protein.

To determine the effect of an exogenous substrate on endogenous metabolism, totally labeled cells were allowed to respire in the absence and in the presence of unlabeled glucose. The cells, supernatant, and CO_2 were analyzed for radioactivity during the time when exogenous glucose was being metabolized (30 min) and after the rate of exogenous oxygen uptake had approached

TABLE 6. *Oxidation of intracellular labeled amino acids by Staphylococcus aureus*

Sample, time	Cells labeled with*			
	U-C^{14}-glutamate	U-C^{14}-alanine	U-C^{14}-glycine	U-C^{14}-aspartic acid
Cells, zero	83.77	110.35	71.89	102.37
CO_2, zero	12.39	3.43	1.41	27.65
CO_2, 100 min	70.0	19.11	3.85	82.6
Assimilated amino acid oxidized, %	83.5	16.4	5.3	80.4

* Unlabeled cells were incubated in Gale's diffusion salts, washed, and placed in Warburg cups. Results are expressed as mμc.

the endogenous level (75 min). The activities at 30 min for the cells not oxidizing glucose were: cells, 46.19 mμc; CO_2, 0.55 mμc; supernatant, 1.75 mμc. For cells oxidizing glucose, the values were 44.51, 0.57, and 1.92, respectively. Values obtained for endogenous cells at 75 min were 44.51, 0.97, and 1.92; values for cells oxidizing glucose were 46.19, 0.94, and 2.42. From these results it is concluded that exogenous glucose does not inhibit endogenous respiration.

To determine if free amino acids other than glutamate could serve as endogenous substrates, unlabeled cells were incubated for 1 hr in Gale's diffusion mixture containing a single labeled amino acid. As pointed out by Blumenthal et al. (1957), such cells are only incompletely labeled. Indeed, under the conditions used here, 84% of the total cellular activity was found in the free pool fraction. Therefore, measurement of labeled CO_2 under these conditions is a qualitative indication of ability to oxidize the free intracellular

amino acid in question. The amino acids chosen were alanine, glycine, glutamate, and aspartic acid. Of the four amino acids tested, glutamate and aspartic acid were oxidized at rapid rates, alanine was oxidized at a much slower rate, and glycine was oxidized slowest of all (Table 6).

Attempts to obtain cells with labeled carbohydrate fractions by growing the organisms in broth supplemented with labeled glucose were unsuccessful because the label did not accumulate intracellularly to an extent which permitted experimentation with labeled cells. In a typical experiment, 168 mg of cells, harvested from 100 ml of broth containing 10 μc of U-C^{14}-glucose, possessed a total activity of only 5 mμc. The resultant activity of liberated CO_2 and cell fractions was so low as to be beyond the accurate sensitivity of the detection instrument. These results would suggest that *S. aureus* does not assimilate glucose but utilizes the compound almost entirely in oxidative processes. This confirms the work of Friedemann (1939), who found that 95% of the glucose was converted to lactic acid and ethanol, and Powelson, Wilson, and Burris (1947), who noted that *S. aureus* assimilates no glucose during the period of active proliferation.

DISCUSSION

The results presented herein indicate that the endogenous respiration of *S. aureus* is enhanced when the organism is grown in the presence of complex substances such as yeast extract. The concentration of amino acids in the medium, rather than the concentration of carbohydrate, also exerts a direct effect on resultant endogenous respiration rate. These results are in contradistinction to those obtained with *Streptococcus mastitidis* (Wood and Gunsalus, 1942), where a medium containing a high nitrogen content and low sugar content resulted in cells with a low endogenous respiration. It has often been assumed that the endogenous substrate is either carbohydrate or lipid in nature (Reiner et al., 1949; Wilkinson, 1959). For example, Stier and Stannard (1936*a*) found an RQ of 1.0 for the endogenous respiration of yeast, and Bernheim (1954) noted that azide and dinitrophenol, which characteristically uncouple oxidation from phosphorylation, also increase the endogenous respiration of *Mycobacterium tuberculosis*. In the present study, an RQ value of 0.83 to 0.86 was observed, and the effect of azide and dinitrophenol on endogenous respiration was found to be negligible.

The data obtained in the present study suggest that several fractions of the cell can serve as sources of endogenous substrates. In particular, the free amino acid pool and protein have been implicated. These results are in agreement with those of Dawes and Holms (1958), who found that the endogenous substrate of *Sarcina lutea* was not carbohydrate in nature and who demonstrated a direct relationship between the rate of endogenous respiration and the level of the free amino acid pool. Similar conclusions were reached by Warren et al. (1960), working with *P. aeruginosa*, and by Bardi and Boretti (1958), studying *N. rugosa*. It should also be noted that Takagaki, Hirano, and Tsukada (1957), working with brain slices, correlated endogenous respiration with the disappearance of intracellular glutamic acid and the appearance of extracellular ammonia. Exogenous glucose inhibited oxidation of endogenous glutamate. However, the concentration of exogenous glucose was high (0.2%) and the experiment was terminated at 1 hr. It is possible that glucose oxidation was not complete and oxidation of intracellular glutamate may have recommenced. Results presented here have demonstrated the resumption of ammonia liberation after exhaustion of exogenous glucose. Also, totally labeled cells were found to liberate labeled CO_2 (and other unidentified labeled compounds) at equal rates during endogenous respiration and while actively oxidizing exogenous glucose.

ACKNOWLEDGMENTS

This investigation was supported by a grant from Research Corporation and a grant (E-1680) from the National Institute of Allergy and Infectious Diseases, U. S. Public Health Service.

LITERATURE CITED

BARDI, U., AND G. BORETTI. 1958. Osservazioni sul metabolismo endogeno di un *Proactinomyces*: *Nocardia rugosa*. Giorn. microbiol. **6**:91–102.

BARKER, H. A. 1936. The oxidative metabolism of the colorless alga, *Prototheca zopfii*. J. Cellular Comp. Physiol. **8**:231–250.

BERNHEIM, F. 1954. Effect of azide and cyanide on the respiration of a species of *Mycobacterium*. Science **120**:430–431.

BLOCK, R. J. 1952. Paper chromatography, p. 67. Academic Press, Inc., New York.

BLUMENTHAL, H. J., H. KOFFLER, AND E. C. HEATH. 1957. Biochemistry of filamentous fungi. V. Endogenous respiration during concurrent metabolism of exogenous substrates. J. Cellular Comp. Physiol. **50**:471–497.

COCHRANE, V. W., AND M. GIBBS. 1951. The metabolism of species of *Streptomyces*. IV. The effect of substrate on the endogenous respiration of *Streptomyces coelicolor*. J. Bacteriol. **61**:305–307.

DAWES, E. A., AND W. H. HOLMS. 1958. Metabolism of *Sarcina lutea*. III. Endogenous metabolism. Biochim. et Biophys. Acta **30**: 278–293.

DOUDOROFF, M. 1940. The oxidative assimilation of sugars and related substances by *Pseudomonas saccharophila*. Enzymologia **9**:59–72.

EATON, N. R. 1960. Endogenous respiration of yeast. I. The endogenous substrate. Arch. Biochem. Biophys. **88**:17–25.

FRIEDEMANN, T. E. 1939. The carbohydrate metabolism of *Staphylococcus aureus*. J. Biol. Chem. **130**:61–65.

GALE, E. F. 1947. The assimilation of amino acids by bacteria. 1. The passage of certain amino acids across the cell wall and their concentration in the internal environment of *Streptococcus faecalis*. J. Gen. Microbiol. **1**:53–76.

GRONLUND, A. F., AND J. J. R. CAMPBELL. 1961. Nitrogenous compounds as substrates for endogenous respiration in microorganisms. J. Bacteriol. **81**:721–724.

MARINO, R. J., AND C. E. CLIFTON. 1955. Oxidative assimilation in suspensions and cultures of *Hydrogenomonas facilis*. J. Bacteriol. **69**: 188–192.

MIDWINTER, G. G., AND R. D. BATT. 1960. Endogenous respiration and oxidative assimilation in *Nocardia corallina*. J. Bacteriol. **79**:9–17.

NORRIS, F. C., J. J. R. CAMPBELL, AND P. W. NEY. 1949. The intermediate metabolism of *Pseudomonas aeruginosa*. I. The status of endogenous respiration. Can. J. Research **27c**: 157–164.

PARK, J. T., AND R. HANCOCK. 1960. A fractionation procedure for studies of the synthesis of cell-wall mucopeptide and of other polymers in cells of *Staphylococcus aureus*. J. Gen. Microbiol. **22**:249–258.

POWELSON, D. M., P. W. WILSON, AND R. H. BURRIS. 1947. Oxidation of glucose, glycerol, and acetate by *Staphylococcus aureus*. Biochem. J. **41**:486–491.

QUASTEL, J. H., AND M. D. WHETHAM. 1924. The equilibria existing between succinic, fumaric and malic acids in the presence of resting bacteria. Biochem. J. **18**:519–534.

RAMSEY, H. H., AND J. L. PADRON. 1954. Altered growth requirements accompanying chloramphenicol resistance in *Micrococcus pyogenes* var. *aureus*. Antibiotics & Chemotherapy **4**:537–545.

REINER, J. M., H. GEST, AND M. D. KAMEN. 1949. The effect of substrates on the endogenous metabolism of living yeast. Arch. Biochem. **20**:175–177.

SHETLAR, M. R., J. V. FOSTER, AND M. R. EVERETT. 1948. Determination of serum polysaccharides by the tryptophane reaction. Proc. Soc. Exptl. Biol. Med. **67**:125–130.

STIER, T. J. B., AND J. M. STANNARD. 1936*a*. A kinetic analysis of the endogenous respiration of bakers' yeast. J. Gen. Physiol. **19**:461–477.

STIER, T. J. B., AND J. M. STANNARD. 1936*b*. The metabolic systems involved in dissimilation of carbohydrate reserves in bakers' yeast. J. Gen. Physiol. **19**:478–494.

TAKAGAKI, G., S. HIRANO AND Y. TSUKADA. 1957. Endogenous respiration and ammonia formation in brain slices. Arch. Biochem. Biophys. **68**:196–205.

UMBREIT, W. W., R. H. BURRIS, AND J. F. STAUFFER. 1957. Manometric techniques, 3rd ed., Burgess Publishing Co., Minneapolis.

VAN NIEL, C. B., AND A. L. COHEN. 1942. On the metabolism of *Candida albicans*. J. Cellular Comp. Physiol. **20**:95–112.

WARREN, R. A. J., A. F. ELLS, AND J. J. R. CAMPBELL. 1960. Endogenous respiration of *Pseudomonas aeruginosa*. J. Bacteriol. **79**: 875–879.

WIAME, J. M., AND M. DOUDOROFF. 1951. Oxidative assimilation by *Pseudomonas saccharophila* with C^{14} labeled substrates. J. Bacteriol. **62**:187–193.

WILKINSON, J. F. 1959. The problem of energy-storage compounds in bacteria. Exptl. Cell Research, Suppl. **7**:111–130.

WILNER, B., AND C. E. CLIFTON. 1954. Oxidative assimilation by *Bacillus subtilis*. J. Bacteriol. **67**:571–575.

WOOD, A. J., AND I. C. GUNSALUS. 1942. The production of active resting cells of streptococci. J. Bacteriol. **44**:333–341.