METABOLIC ASPECTS OF BACTERIAL GROWTH IN THE ABSENCE OF CELL DIVISION

II. Respiration of Normal and Filamentous Cells of Bacillus cereus[1]

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Received for publication April 18, 1952

Filamentous forms of rod-shaped bacteria may be seen occasionally in cultures under ordinary conditions and may be induced to occur in abundance under a variety of experimentally controlled conditions. Hansen (1894) showed that incubation at elevated temperatures (40.5 to 41.0 C) gave rise to completely filamentous populations in the films formed by species in the genus *Acetobacter* on weakly alcoholic media. Cernovodeanu and Henri (1910) demonstrated that ultraviolet irradiation of gram positive rod-shaped bacteria gave rise to a completely filamentous population of rods with greatly lessened gram positivity. More recently, filamentation of rod-shaped bacteria has been shown by many workers to result from the application of antibiotics to susceptible cultures. George and Pandalai (1948) demonstrated a loss of gram positivity accompanying penicillin induced filamentation of gram positive rods. The critical importance of the magnesium content of the growth medium in governing the cell length in species of the genus *Clostridium* has been analyzed in a series of papers by Webb (1948, 1951). From a study of the effects of a variety of inhibitory substances on *Aerobacter aerogenes*, Hinshelwood (1946) has concluded that filament formation in this rod-shaped organism is the result of an imbalance in the rates of growth and of cellular division. Nickerson (1948) has considered the general validity of this hypothesis as an explanation for filament formation by bacteria and yeasts.

Despite the number of years during which the existence of filamentous forms of bacteria has been known, there has been no report comparing the metabolism of elongated cells with normal cells of the same strain. This has resulted from the difficulties involved in obtaining homogeneous populations of elongated cells. With the magnesium removal procedure of Webb, it is possible now to obtain good yields of uniformly elongated cultures of rod-shaped bacteria with cells 20 to 50 μ in length. The first paper in this series (Nickerson, 1950) compared

[1] Supported in part by a grant from the American Cancer Society, made upon the recommendation of the Committee on Growth of the National Research Council.

[2] This work was initiated during the author's tenure as Visiting Professor at Brown University; he wishes to thank Professor J. Walter Wilson, Director of the Arnold Biological Laboratories, and Professor Charles A. Stuart for the many courtesies they extended to him.

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the pentose nucleic acid contents of filamentous and normal cells of *Bacillus cereus*; no diminution of analytically detectable pentose nucleic acid (on a unit weight basis) was observed to accompany growth in the absence of cellular division. The lowering of affinity for basic dyes which was characteristic of elongated cells of *B. cereus* thus could not be explained on the grounds of lessened nucleic acid content.

It is well known that a variety of conditions, inhibitory to microbial growth, may be conducive to the development of some filamentous cells in cultures. It must be emphasized, however, that the phenomenon of growth in the absence of cellular division is concerned, not with such growth-limiting situations, but with selective inhibition of cellular division in a manner whereby growth is scarcely hindered. In the situation specified, the vast complex of metabolic reactions associated with growth continues, while the reactions associated with cellular division suffer some block. Through a comparative study of metabolic reactions of normal cells and elongated cells, one is provided with a means of investigating the mechanism of cellular division in microorganisms.

This paper is concerned with the question of the energy supply available to an aerobic organism in a physiological state permitting both growth and cellular division and to the organism in a state permitting growth in the absence of cellular division.

MATERIALS AND METHODS

The work was carried out with one strain[4] of *B. cereus*, strain C5-25, which was maintained in the culture collection of the Bacteriology Laboratory of Brown University. Cultures designated as "normal" or "short cell" were obtained by incubation for 18 hours in 2 per cent Evans peptone broth with continuous agitation at 37 C. Cultures consisting almost exclusively of filamentous cells of 20 to 50 μ length were obtained after incubation for the same time and at the same temperature in a magnesium deficient medium prepared by the procedure of Webb (1948). Conventional manometric procedures were employed to study oxygen consumption by washed suspensions of filamentous or normal cells. Total nitrogen was determined by a micro-Kjeldahl method.

RESULTS

Growth and nitrogen content of normal vs. elongated cells. In table 1 are given values from six representative experiments for culture density, dry weight per unit volume of culture, and total nitrogen of the two cell types. All values are averages of determinations on duplicate samples. These data show that the total amount of growth of cells in which division had been selectively inhibited by magnesium deficiency was the same as the growth of cells with an unimpaired division mechanism. The total nitrogen content of the two types of cells in any experiment has been the same within the limits of the method of determination.

Oxygen consumption of normal vs. elongated cells. Values for the rate of oxygen

[4] The authors wish to thank Dr. Ruth E. Gordon for verifying the identification of this organism.

consumption by filamentous and by normal cells on a variety of substrates are tabulated from seven experiments in table 2. The rate of oxygen consumption of the two cell types in the absence of added substrate (endogenous) has been approximately the same in all experiments; a mean Q_{O_2} value of 7.8 has been obtained for the filamentous type and a mean of 9.3 for normal cells.

On the addition of glucose, pyruvate, glutamate, or alanine to the normal cells an immediate increase of 7 to 10 fold occurs in the rate of oxygen consumption. These substrates are oxidized at a much slower rate by the filamentous cells;

TABLE 1

Optical density, dry weight, and total nitrogen content of filamentous and of normal cells of Bacillus cereus

EXP NO. AND CELL TYPE	OPTICAL DENSITY OF CULTURES	DRY WEIGHT OF CELLS, MG/ML	TOTAL NITROGEN OF CELLS	
	Log I_0/I		mg/ml	% of dry wt
8–29				
Short	0.312	0.26	0.030	11.9
Long	0.352	0.33	0.033	10.0
8–31				
Short	0.321	0.43	0.044	10.4
Long	0.314	0.40	0.048	12.2
9–11				
Short	0.299	0.36	0.045	12.6
Long	0.280	0.31	0.038	12.4
9–13				
Short	0.297	0.33	0.044	13.4
Long	0.299	0.29	0.040	14.1
9–18				
Short	0.299	0.41	0.044	10.8
Long	0.292	0.34	0.037	10.7
9–20				
Short	0.305	0.41	0.049	12.1
Long	0.305	0.35	0.042	12.2

glucose and alanine being oxidized at about $\frac{1}{6}$ the rate found with normal cells, and pyruvate and glutamate at about $\frac{1}{3}$ the rate for short cells. Acetate was not oxidized by either cell type and succinate prompted only a 2 to 3 fold increase in oxygen uptake by either cell type. The time courses for oxidation of the various substrates are shown in figures 1 to 3.

Effect of substrate concentration on oxygen consumption. The difference between the rate of oxidation of glucose by filamentous and by normal cells cannot be explained on the basis of a higher substrate concentration requirement of the long cells. Data in table 3 show that the minimum substrate concentration per-

mitting a constant rate of oxygen consumption is lower for elongated cells than for normal cells. This experiment also indicates that substrate concentration is not limiting the rate of uptake of substrate by the elongated cells.

In one experiment long and short cells were incubated for three hours at 30 C in the presence of magnesium, calcium, or ferric ions before readings of oxygen consumption were begun. These additions were without effect on the oxidation of glucose by either type of cell.

TABLE 2

Oxidation of various substrates by filamentous and by normal cells of Bacillus cereus

EXP NO. AND CELL TYPE	ENDOGENOUS	GLUCOSE	ACETATE	PYRUVATE	SUCCINATE	GLUTAMATE	ALANINE
11–25							
Short	7.9	80.3					
Long	9.3	14.3					
12–20							
Short	8.8	71.5				57.5	
Long	7.6	16.7				24.4	
8–29							
Short	14.0	86.5					
Long	10.5	49					
8–31							
Short	9.8	65		78			
Long	8.2	20.5		27			
9–11							
Short	7.2	42	8.5				
Long	6.1	3.1	5.3				
9–13							
Short	8.5	65					
Long	7.4	10.2					
9–20							
Short	8.4				21		95.0
Long	5.4				10.5		12.2

Effect of respiratory poisons on oxygen consumption by elongated cells. The lower rate of oxidation of glucose and other substrates by the filamentous cells may represent a generalized lowering of the over-all efficiency of the respiratory process. This could result, for example, from decrease in the number of enzyme bearing surfaces per unit dry weight or from a lowering of the rate of entry of substrate as a result of the decrease in surface:volume ratio. The possibility that the decrease in rate is not due to a general cause, but is the result of the elimination of some one component from a multiple pathway respiratory mechanism, must also be considered. Limitation of the Q_{O_2} in long cells by diffusion

of substrate into the cell is somewhat unlikely in view of the experiment cited in table 3 wherein the substrate concentration was varied from 5×10^{-4} M to 10^{-2} M. Through the use of respiratory inhibitors with some specificity of action, it is possible that one may be able to decide if similar enzyme systems are operating in the cells which have been growing and dividing normally and those which have been growing only. Experiments were conducted in which fluoride, iodo-

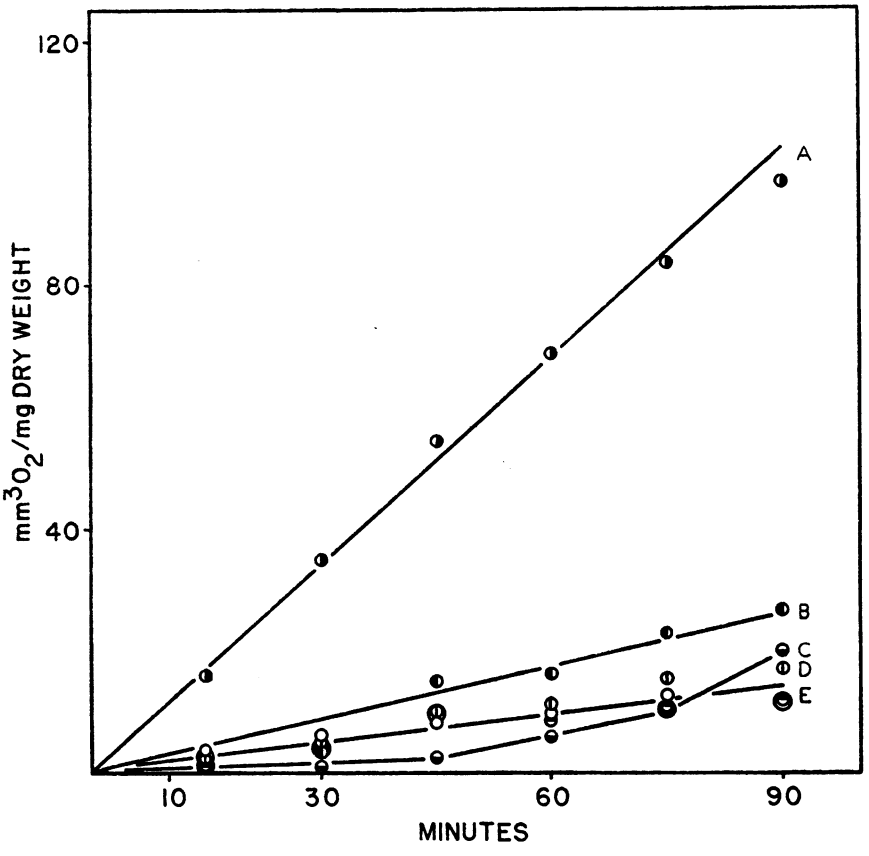

Figure 1. Oxygen consumption by filamentous and normal cells of *Bacillus cereus*. Curve A, normal cells with glucose; curve B, long cells with glucose; C, long cells with acetate; D, long cells—endogenous respiration; E, short cells—endogenous respiration and short cells with acetate.

acetic acid, or urethane was added to filamentous and to normal cells metabolizing glucose.

Iodoacetate. As shown in figure 4 the endogenous and exogenous respiration of both short and long cells is inhibited in similar fashion by 7×10^{-4} M iodoacetate. The low rates of oxygen consumption by short and long cells, either with or without added substrate, are essentially identical following the addition of iodoacetate. The iodoacetate-insensitive respiration of long cells comprises

a somewhat larger proportion of their total respiration than does the comparable fraction in short cells. In both cell types the iodoacetate-insensitive respiration is responsible for equivalent amounts of oxygen consumption per unit dry matter. It may be concluded that the respiratory mechanism which is lost to long cells is part of the iodoacetate-sensitive fraction.

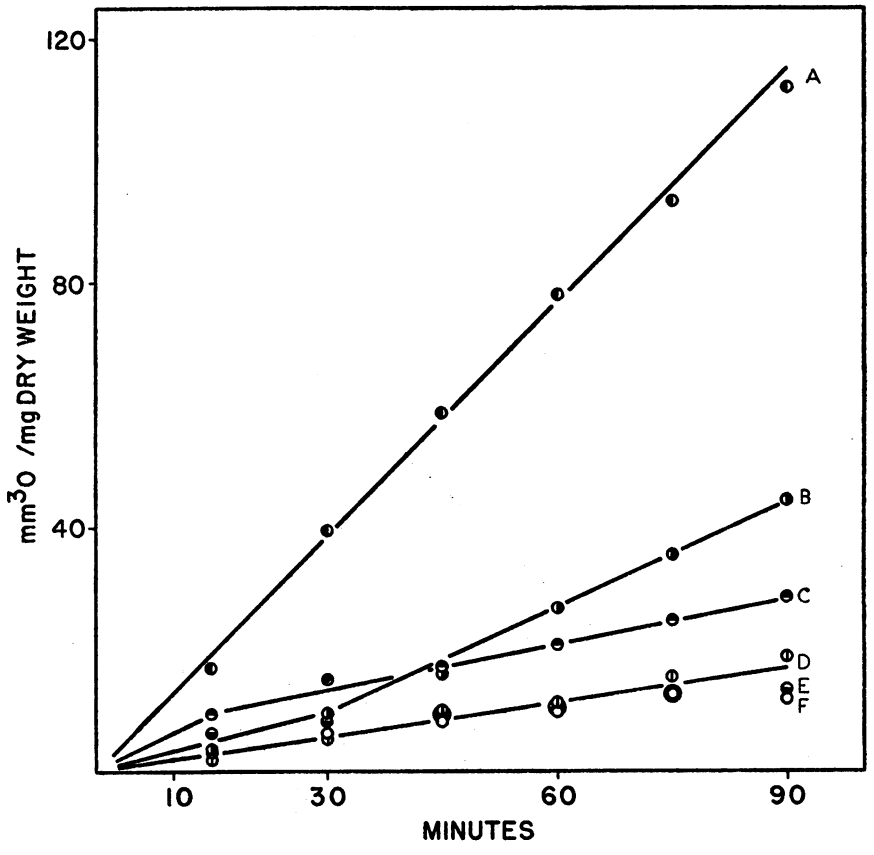

Figure 2. Effect of pyruvate and succinate on the oxygen consumption of normal and filamentous cells of *Bacillus cereus*. Curve A, short cells with pyruvate; curve B, long cells with pyruvate; C, short cells with succinate; D, endogenous respiration of short cells; E and F, long cells with succinate and endogenous respiration of long cells.

Fluoride. The effect of fluoride on the respiration of long and short forms was examined in a few experiments. Oxygen consumption by the normal form of *B. cereus* is not inhibited by fluoride except at concentrations of NaF greater than M/100, whereas the exogenous oxidation of glucose by long cells is completely suppressed by 7×10^{-3} M NaF (figure 5). The apparently increased sensitivity of long cells to fluoride may be the result of their greater permeability to fluoride since the short cells, after an exposure of 1 hour to NaF, exhibited a decline in their rate of oxidation of glucose. The decline in rate could not be explained on the basis of incomplete oxidation of the substrate. The fluoride effect

which we have observed may also be considered in relation to the work of Runnström, Gurney, and Sperber (1941) on the protection against NaF afforded to

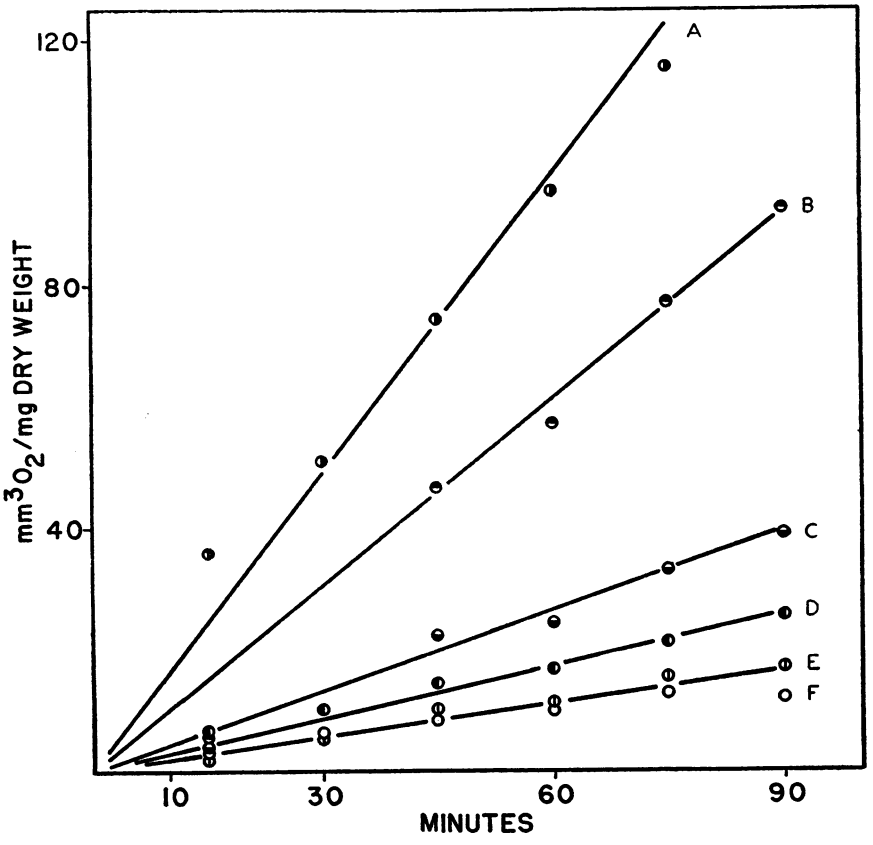

Figure 3. Oxidation of amino acids by normal cells and by filamentous cells of *Bacillus cereus*. Curves A and B, short cells with alanine or glutamate; C and D, filamentous cells with glutamate or alanine; E and F, endogenous respiration of long and short cells.

TABLE 3

Effect of glucose concentration on oxygen consumption of filamentous and of normal cells of Bacillus cereus

Data as mm^3 O_2/mg dry wt/hr

CELL TYPE	CONCENTRATION OF GLUCOSE			
	Zero	5×10^{-4} M	5×10^{-3} M	10^{-2} M
Short	8.5	43.5	65	65
Long	7.4	9.3	6.9	10.2

respiring yeast cells by the presence of metabolizable substrates. A study of the basis for the difference in effect of fluoride on long and on short cells is in progress.

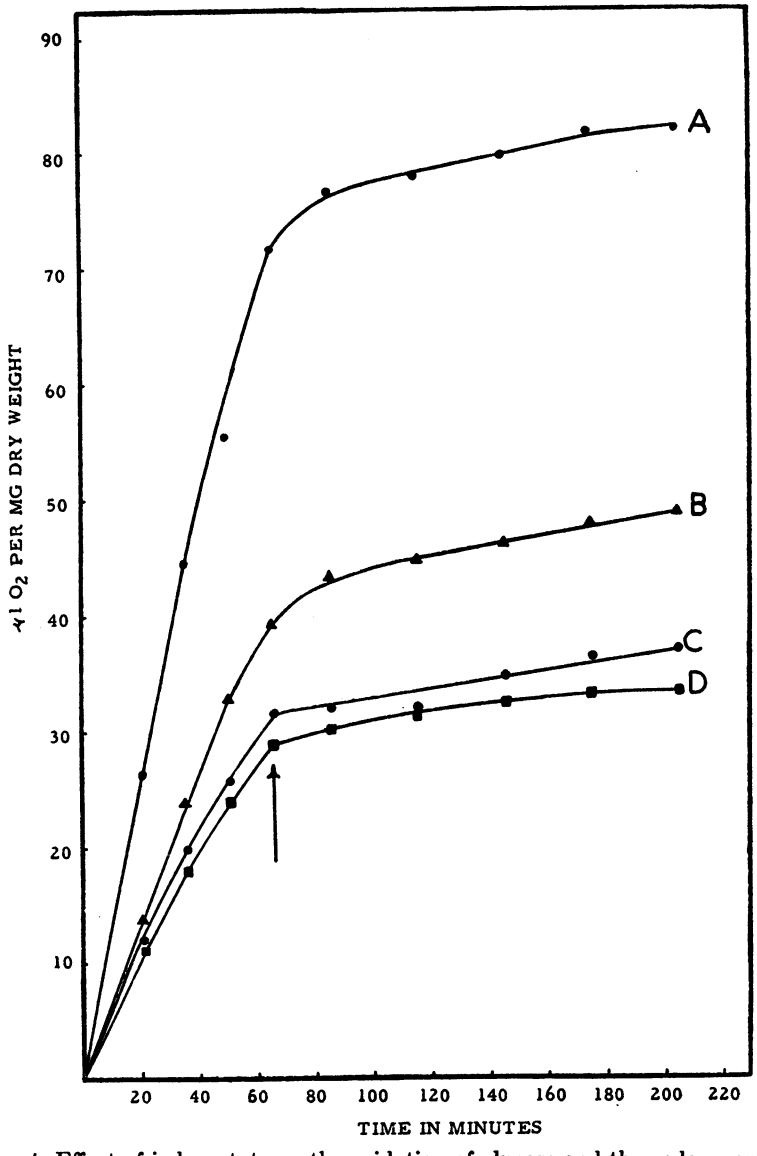

Figure 4. Effect of iodoacetate on the oxidation of glucose and the endogenous respiration of normal and filamentous cells of *Bacillus cereus*. Curve A, short cells with glucose; curve B, filamentous cells with glucose; curves C and D, endogenous respiration of filamentous and normal cells. At arrow, iodoacetate added to give final concentration of 7×10^{-4} M.

Urethane. Fisher and Stern (1942) and Fisher (1942) demonstrated that plots of concentration of urethane vs. oxygen consumption (by resting cells of bakers' yeast) exhibited a discontinuity at high concentrations of urethane (0.2 to 0.25 M). At this level of urethane concentration an inhibitory effect on cellular multiplication became pronounced. They assumed that cellular multiplication in

bakers' yeast required the integrity of a "more susceptible" respiratory system inhibited by urethane.

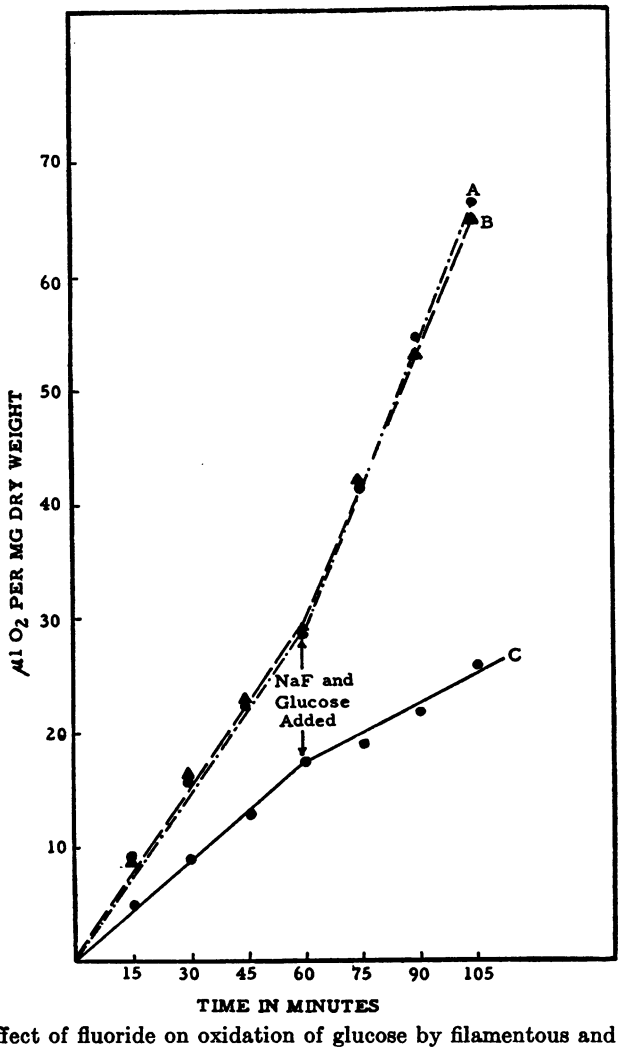

Figure 5. Effect of fluoride on oxidation of glucose by filamentous and normal cells of *Bacillus cereus*. Curve A, normal cells with glucose; curve B, normal cells with glucose and fluoride; curve C, long cells with glucose and fluoride. At arrow, glucose added to all and NaF added to B and C to give final concentration of 7×10^{-3} M.

The response of the long cell type of *B. cereus* to low concentrations of urethane (ethyl N-ethyl carbamate) is different from that of the short forms. As seen in figure 6 (C and D) oxygen consumption by long forms is markedly stimulated by urethane at 1.5 to 3.0×10^{-3} M. This is, almost exclusively, a stimulation of the endogenous respiration (compare figure 6C and D with E). With short cells (figure 6A and B) these concentrations of urethane bring about a very slight

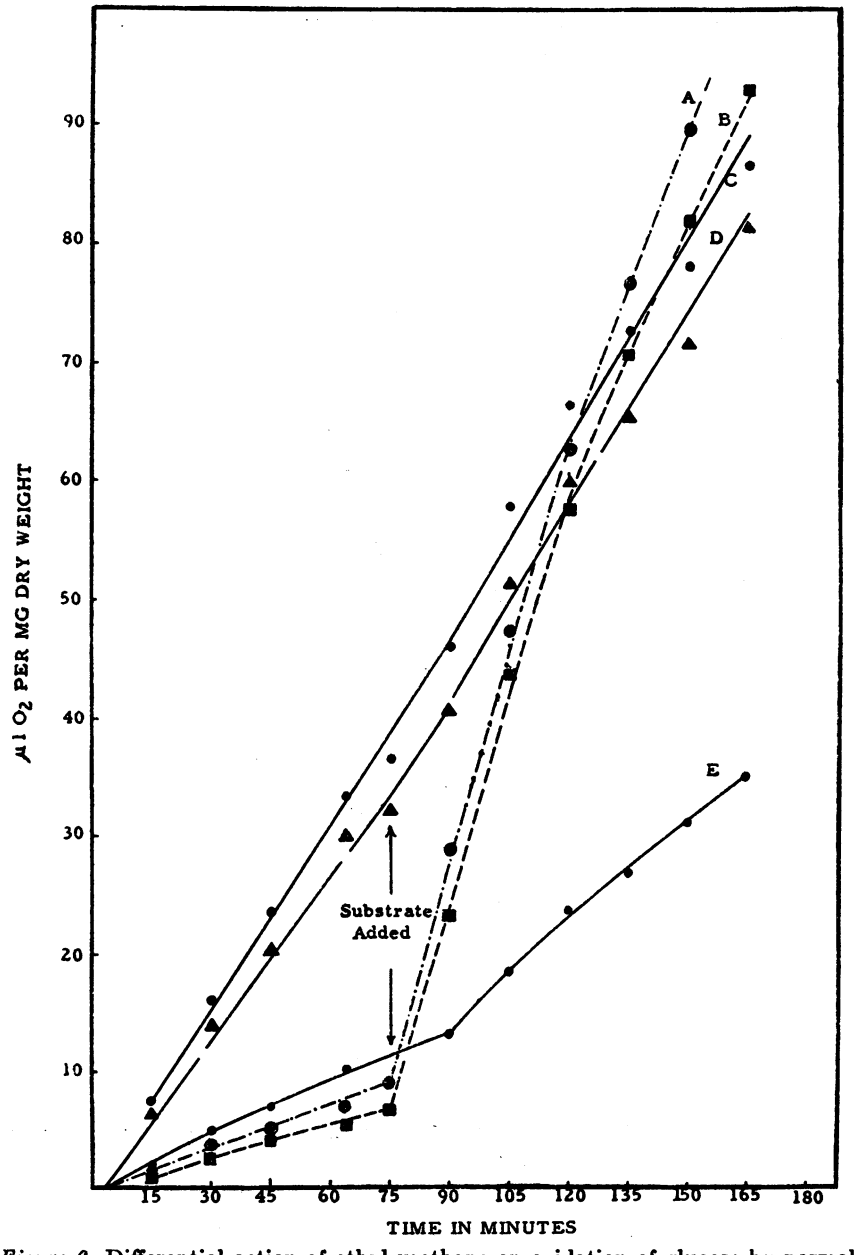

Figure 6. Differential action of ethyl urethane on oxidation of glucose by normal and filamentous cells of *Bacillus cereus*. Curves A and B, normal cells; A, with 3×10^{-3} M urethane; B, without urethane. Curves C, D, and E, filamentous cells; C, with 3×10^{-3} M urethane; D, with 1.5×10^{-3} M urethane; E, without urethane. At arrow, glucose added to all.

stimulation of endogenous and exogenous respiration. The exogenous respiration of short cells does not fall off to the endogenous levels (due to substrate exhaus-

tion) so rapidly in the presence of low concentrations of urethane as it does in the short cell controls. This may indicate that the action of dilute urethane on both cell types is directed at the oxidation of reserve stores. A study of the amount and composition of reserve carbohydrate stores in long cells and normal cells of *B. cereus* will be reported later.

DISCUSSION

The low rates of oxygen consumption exhibited by filamentous cells of *B. cereus* with a variety of substrates indicate that energy yielding reactions may be factors limiting cellular division in these forms. In view of the fact that the long cell types have (with pyruvate) but ⅓ of the rate of exogenous respiration of normal cells, one might say that growth requires only ⅓ of the total, normal rate of oxygen consumption exhibited by short cells, whereas the process of cellular division necessitates energy utilization from ⅔ of the total of oxidative processes. This interpretation is analogous to the concept of "activity metabolism" developed by Fisher and Stern (1942). Their view that inhibition of cellular multiplication in yeast was closely correlated with inhibition of a defined fraction of the total respiration receives support from our study.

The data presented on rates of synthesis of cell mass by the long and short cell forms, taken in conjunction with the data on the nitrogen content of the two cell types, are definitive evidence that the amount of cellular growth (made in 16 hours) is the same (in this organism) whether or not it is accompanied by cellular division. This is, furthermore, evidence that cellular division is not an essential accompaniment of cellular growth and is proof that the division mechanism may be uncoupled from growth processes without impairment to the latter function. The constancy of the nitrogen content of the two types is an indication that the rate of protein synthesis is unaffected by the selective inhibition of cellular division (accomplished by control of the magnesium content of the growth medium).

The energy requirement for the syntheses associated with growth is met by a small fraction of the total normally made available by the oxidative metabolism of the multiplying cell. Of the total exogenous respiration of these bacterial cells, the fraction associated with the support of growth is almost completely inhibited by iodoacetate and is remarkably sensitive to fluoride.

SUMMARY

The magnesium-deficiency technique of Webb has been employed to obtain crops of *Bacillus cereus* in the form of long filaments. Determinations of culture turbidity and dry weight of crops indicate that filamentous cells and normal cells achieved the same total growth in 16 hours of incubation. The total nitrogen content of filamentous cells per unit weight was the same as that of normal cells. Rates of oxygen consumption in the absence of added substrate were compared for normal and filamentous cells and found to be similar. Oxidations of glucose, pyruvate, alanine, and glutamate by filamentous cells proceeded at rates only ⅓ to ⅙ of those exhibited by cells with uninhibited division mechanisms. The fraction of the total respiration retained by growing nondividing

(filamentous) cells is almost completely inhibited by iodoacetate and is sensitive to fluoride. Implications derived from this aspect of the study of the metabolism of bacteria grown in the absence of cell division are considered.

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