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Transient Responses of Glucose-Limited Cultures of *Cytophaga johnsonae* to Nutrient Excess and Starvation

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Cells from glucose-limited chemostat cultures of *Cytophaga johnsonae* were subjected to a sudden relaxation of substrate limitation by injecting the cells into fresh batch cultures. Starvation experiments were carried out by injecting glucose-limited cells into batch cultures lacking glucose. Transient responses of biomass, glucose uptake and mineralization, ATP content, and viability on different agar media were monitored during these nutrient-shift experiments. Cells reacted differently depending on growth rate and time spent in the chemostat. Fast-growing cells showed an immediate adaptation to the new growth conditions, despite some initial overshoot reactions in ATP and uptake potential. In contrast, slowly growing cells and long-term-adapted cells showed extensive transient growth responses. Glucose uptake and mineralization potentials changed considerably during the transient growth phase before reaching new levels. During the starvation experiments, all cell types displayed a fast decrease in ATP, but the responses of the substrate uptake and mineralization potentials were strongly dependent upon the previous growth rate. Both potentials decreased rapidly in cells with high growth rates. On the other hand, cells with low growth rates maintained 80% of their uptake and mineralization potentials after 8 h of starvation. Thus, slowly growing cells are much better adapted for starvation than are fast-growing cells.

Transient growth responses of microorganisms can be defined as the growth responses of a microbial population in steady state, or balanced growth, to a disturbance. Transient responses extend until a new equilibrium has been reached. These transient responses are interesting from an ecological, physiological, and applied point of view. Ecologically, most microbial communities are exposed to continual changes in physical and chemical environments, which in turn, induce changing growth responses (11). In physiological studies, the power of transient growth kinetics for the investigation of dynamic processes like protein, DNA, and RNA syntheses was shown in the classic study of Maaloe and Kjeldgaard (19). Applied aspects of transient growth responses concern the loading of sewage plants as well as the discontinuous operation of large fermenters (4, 22).

In aquatic habitats, microorganisms are subjected to diel cycles (15, 21), micropatchiness (17), and many other factors responsible for changes in the substrate concentration (6). The present study focuses on eco-physiological responses of a homogeneous bacterial population to changed nutritional situations. These responses were studied with glucose-limited chemostat cultures grown at different rates and after different adaptation times to a steady state. Instead of changing from one dilution rate to another, an approach often used and recently summarized by Cooney et al. (3), in the present study, bacteria were subjected to sudden extreme nutritional situations: i.e., either to nutrient excess or to starvation. This approach results in more-clear-cut information regarding adaptation times and growth kinetics than that obtained by a shift of the dilution rate. To measure growth responses of slow- and fast-growing bacterial populations, cells from glucose-limited chemostat cultures were removed from the reactor and injected into a fresh batch culture either with (shift-up experiment) or without (shift-down experiment) glucose in the mineral medium. Because cells adapted to the chemostats for longer terms showed quite different features from short-term-adapted cells (10), the effect of the time length at a steady state on transient responses was investigated, as was the influence of different growth rates.

The gliding, heterotrophic, freshwater bacterium, *Cytophaga johnsonae*, was chosen as the test organism. This bacterium possesses two different glucose uptake systems at high and low glucose concentrations and may be a representative of versatile autochthonous aquatic bacteria which are facultatively oligotrophic (2, 5, 9). Glucose uptake and mineralization rates were measured during transient growth experiments to describe the adaptation of the substrate turnover potential of this bacterium. The ATP content of cells and the number of CFU were also monitored to determine the responses of the energy metabolism and the viability of the cells.

MATERIALS AND METHODS

Organism. *C. johnsonae* C-21, a freshwater isolate with psychrotrophic growth characteristics, has been described previously (8, 9).

Media. All liquid culture experiments were done in a defined, phosphate-buffered, mineral medium (8) containing 450 mg of glucose per liter (2.5 mmol/liter) as the sole organic carbon and energy source. The medium was sterilized as a whole by membrane filtration after adjusting the pH to 7.1 with 1 mol of sodium hydroxide per liter. Two agar media were used for plate counts. (i) G2 agar: the phosphate buffer of the mineral medium (8) was autoclaved with 15 g of agar (no. 1614; E. Merck AG) per liter and filter-sterilized glucose (2 g/liter); the remainder of the salts of the mineral medium were added afterwards. (ii) N8 agar consisted of 8 g of nutrient broth (no. 5443; Merck) and 15 g of agar per liter in distilled water.

Culture conditions. Conical culture tubes (diameter, 45 mm; length, 330 mm) were used for batch cultures. These culture tubes, filled with 250 ml of medium, were aerated at a rate of 25 liters/h. The continuous cultures were carried out

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in similar but larger reaction vessels (diameter, 100 mm; length, 370 mm; Sovirel Corp.) containing 1 liter of medium. The aeration rate was 100 liter/h. The pH of the medium decreased during growth by less than 0.1. Temperature was maintained at 20°C (±0.1°C) by immersing the cultures in a water bath. Details of the construction and operation of the chemostat are given elsewhere (9; M. G. Höfle, Ph.D. thesis, University of Freiburg, Freiburg, Federal Republic of Germany, 1981).

Analyses. Optical densities (OD) were read in a Zeiss Pl 4 spectrophotometer at 578 nm with a 1-cm cuvette. Estimates of dry weight from the OD values were done on the basis of a standard curve. An OD value of 1 was equal to 657 mg [dry wt] per liter. A single, standard curve proved adequate, regardless of the growth rate or culture type (batch or chemostat).

For the ATP analysis, 200 μl of a cell suspension was withdrawn from the culture and injected into 800 μl of boiling, Tris-buffered EDTA and boiled for 3 min to extract cellular ATP (18). After being cooled to room temperature, these extracts were frozen at −20°C until further analysis. The ATP content of the extracts was determined with a Lumacounter 2080 (Abimed GmbH), by using highly purified luciferase (Lumit PM grade; Abimed) dissolved in 25 mmol of HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) buffer per liter (pH 7.75 [Abimed]). The internal standardization technique of Strehler (24) was used for calibration and calculation of the absolute amount of ATP in the extracts.

Plate counts (CFU). The culture suspension was diluted stepwise (100 times) with sterile mineral medium without glucose to estimate the number of CFU. Then, a 100-μl sample of an appropriate dilution was spread on the relevant agar plate and incubated for 7 to 10 days at 20°C. The CFU values were calculated from eight replica plates by the method of Postgate (23) for optimal plate count estimates. To calculate the specific plate count values (CFU/mg [dry wt]), the OD value of the undiluted suspension was used to determine dry weight, as detailed above.

Uptake assay. For all uptake assays, cells were washed on a membrane filter (47-mm diameter, 0.2-μm pore size; Nucleopore Corp.) with sterile mineral medium and suspended in mineral medium to a final concentration of 1 to 2 mg (dry wt) per ml. The uptake assay was started by adding 1 ml of the washed cell suspension to 50 μl of [*U*-^{14}C]glucose solution (specific activity, 1.5 mCi/mg; final assay concentration, 1 μmol/liter; Amersham Corp.). After 7.5, 15, 30, 45, and 60 s, 100 μl of the reaction mixture was placed on a membrane filter (25-mm diameter, 0.65-μm pore size, cellulose nitrate; Sartorius GmbH), filtered immediately, and washed with 8 ml of mineral medium. The absolute uptake rate was calculated from the slope of the least-squares linear regression line for the five counts-per-minute measurements of the membrane filters. For the calculation of the specific uptake rate, biomass was calculated from the OD of the washed cell suspension. All specific uptake rates were measured at 20°C under the above-indicated conditions; they therefore represent potential uptake rates of the cells and not actual uptake rates in the culture. Details of the washing procedure and the uptake measurement are given elsewhere (9; Höfle, Ph.D. thesis).

Mineralization measurement. The CO_2 evolved by the cells during the uptake assay was measured in a sample of the washed cell suspension (see above). The washed cells were incubated under the same conditions for 60 s in a second test tube sealed with a silicon stopper. $^{14}CO_2$ was trapped in a phenethylamine-soaked filter paper as described elsewhere (9). Mineralization rates were calculated by assuming mineralization to be linear from 0 to 60 s.

Shift experiments. The shift-up experiments were done by removing 25 ml of cell suspension from the relevant chemostat culture with a sterile syringe and injecting it into a batch culture tube filled with 250 ml of sterile glucose mineral medium. For the shift-down experiments, the same procedure was used, but the medium did not contain any glucose. All shift experiments were done at least in duplicate, but all figures, values, and percent standard deviations are given only for one representative of the replicates. Because of slight differences in biomass values or because of time shifts in the reaction patterns, data from different culture tubes could not be averaged.

RESULTS

First shift-up experiment. In the first shift-up experiment, fast-growing cells from the steady state of a glucose-limited chemostat culture (D = 0.15 h^{-1}, 75% μ_{max}) were injected into a fresh batch culture. Since the chemostat glucose concentration was 0.14 mmol/liter (9), this shift resulted in an 18-fold increase in the glucose concentration for the cells. The immediate increase of the OD after the shift showed that the cells of *C. johnsonae* grew after the shift without any lag and with the maximum growth rate (μ_{max}) of 0.20 h^{-1} measured in regular batch cultures (Table 1; reference 8).

The glucose uptake and mineralization potential peaked 1 h after the shift-up (Fig. 1a). After this peak, both potentials leveled off at averages of 0.065 (±7.2%) and 0.021 (±25.7%) nmol of glucose per min per mg (dry wt), respectively. Toward the end of the logarithmic growth phase, both potentials started to decrease. Their absolute values during the stationary phase were less than one-third of the logarithmic-phase values. Both the absolute value and the pattern of the substrate turnover potential (except the initial peak) were comparable to the results obtained in an undisturbed batch culture (9).

The cellular ATP content peaked (1.63 ng of ATP per μg [dry wt]) 0.5 h after the shift (Fig. 1b). After 2 h, the ATP content remained constant at an average value of 1.19 (±2.2%) ng of ATP per μg (dry weight) until the end of the logarithmic phase. This average level was not significantly different from the ATP levels measured in the logarithmic phase of a regular batch culture or a fast-growing chemostat culture (Höfle, Ph.D. thesis; 10). The ATP content decreased almost 50% as soon as the culture reached its stationary phase. This was also typical of regular batch cultures (Höfle, Ph.D. thesis).

Specific CFU values were chosen to show changes in the plate counts during the experiments, independent of changes in biomass. The specific CFU values on N8 agar decreased slightly during the first 8 h of the shift experiment (from 2.8 × 10^9 to 2.4 × 10^9 [±12%] cells per mg [dry weight]) and showed an increase during the stationary phase (3.2 × 10^9 [±14%] cells per mg [dry weight]; Fig. 1c). This pattern also occurred in regular batch cultures (Höfle, Ph.D. thesis). The increase in the specific CFU values during the stationary phase actually reflects the decrease in average cell size which was observed microscopically (Höfle, Ph.D. thesis). The specific CFU values were lower (20%) during the first 8 h on G2 agar than on N8 agar. This was also the case in batch and fast-growing chemostat cultures (Höfle, Ph.D. thesis; 10). The only unexpected finding in terms of specific CFU values was that the number of CFU on G2 agar decreased

during the stationary phase. The final value of CFU on G2 agar was only 52% of that on N8 agar.

Second shift-up experiment. In the second shift-up experiment, cells from a slowly growing chemostat culture (D = 0.03 h^{-1}, 12.5% μ_{max}) were injected into a batch culture (Fig. 2a to c). This shift-up resulted in an increase of the substrate concentration for the cells of more than three orders of magnitude, from 1.5 μmol of glucose per liter in the chemostat to 2.5 mmol of glucose per liter in the batch culture (9). As the constancy of the OD values indicates, the cells did not grow for the first hour after the shift. The cells then grew

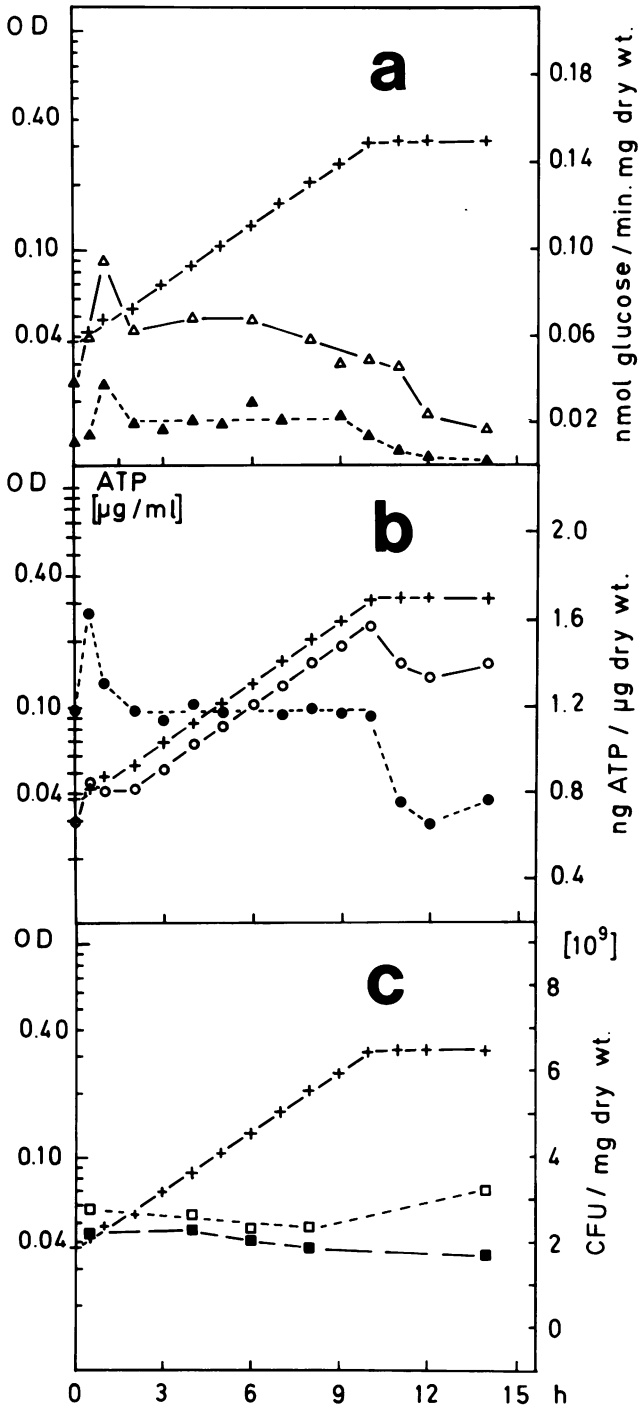

FIG. 1. First shift-up experiment. *C. johnsonae* cells from a fast-growing glucose-limited chemostat culture (D = 0.15 h^{-1}) were injected at 0 h into a fresh batch culture. Symbols: (a) +, OD; △, glucose uptake potential; and ▲, glucose mineralization potential. (b) +, OD; ○, ATP per ml of cell suspension (same scale as OD); ●, ATP per biomass. (c) +, OD; □, specific plate counts on N8 agar, and ■, specific plate counts on G2 agar.

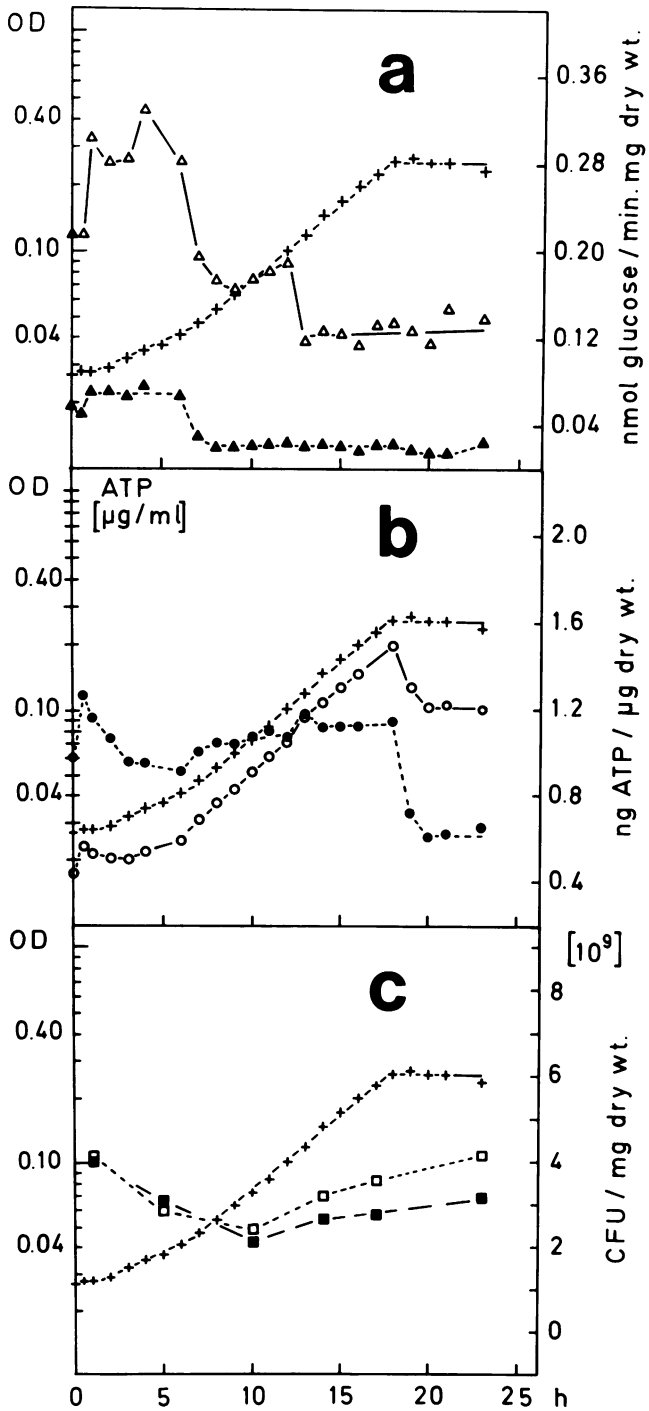

FIG. 2. Second shift-up experiment. *C. johnsonae* cells from a slowly growing glucose-limited chemostat (D = 0.03 h^{-1}) were injected at 0 h into a fresh batch culture. Graphical arrangement and symbols are as in Fig. 1.

exponentially with a growth rate of 0.086 h^{-1} (43% μ_{max}, Table 1) for 6 h; after 7 h, they doubled their growth rate to 0.165 h^{-1} (83% μ_{max}).

Large changes in the glucose uptake and mineralization potential were associated with this triphasic growth response (Fig. 2a). During the first hour, uptake potential increased to an average of 0.3 nmol of glucose per min per mg (dry weight).

This level remained more or less constant until the end of the second growth phase. Glucose uptake and mineralization potential dropped by one-half after 6 h at the end of the second growth phase. During the last growth phase (highest growth rate), the uptake dropped again by 30% after 12 h, but mineralization remained constant. Neither potential showed any significant decrease when the culture reached the stationary phase.

The ATP content of the biomass showed a small peak after 0.5 h and then declined to a minimum of 0.92 ng of ATP per μg (dry weight) near the end of the second growth phase (Fig. 2b). During the third phase, the ATP content rose slightly and was greater than 1 ng of ATP per μg (dry weight). As in the first shift-up experiment, attainment of the stationary phase was again characterized by a 50% decrease in ATP content.

The specific plate counts on both agar media decreased during the first 10 h, mainly owing to an increase in cell size (Fig. 2c). After 10 h, the specific CFU values increased for both media, but unlike the first logarithmic phase, the difference between the N8 and the G2 agar CFU values increased with time.

Third shift-up experiment. In the third shift-up experiment, a bacterial population was used which had been grown at a high growth rate (D = 0.15 h^{-1}) for 1,270 h (275 generations), in contrast to the two preceeding shift experiments in which cells from the first steady state (5 to 20 generations) of a chemostat culture were used (for details, see references 9, 10). These long-term-adapted cells showed a 20-fold greater uptake potential but only 15% of the specific CFU values compared with the cells which were used in the first shift-up experiment (10). The shift-up experiment resulted in a 700-fold increase of the ambient substrate concentration for these cells which had been growing in 3.5 μmol glucose per liter (10).

The growth response of the cells was triphasic as in the second experiment (Fig. 3a). For the first 3 h, the cells grew at a rate of 0.16 h^{-1} (Table 1), i.e., the growth rate was not significantly different from that in the chemostat. In the second phase, the growth rate decreased and growth was nonlogarithmic (3 to 7 h, μ = 0.103 h^{-1}, average logarithmic growth rate for the whole period). In the third growth phase, after 8 h, the cells grew logarithmically again and at their maximum growth rate (Table 1).

The pattern of the other measured parameters showed some correlation to this growth response. Substrate uptake and mineralization potentials stayed high for the first 2 h and decreased drastically toward the end of the first growth phase (Fig. 3a). In the second phase, these parameters were at ca. one-half of their initial values. The third phase, after 8 h, was characterized by a linear decrease in both potentials. The increase of both potentials after the culture reached the stationary phase was unusual.

In the first growth phase, the ATP content of the biomass peaked after 0.5 h at 1.57 ng of ATP per μg (dry weight) and showed a minimum in the middle of the second growth phase (Fig. 3b). The third growth phase was characterized by a constant ATP level at an average of 1.19 ng of ATP per μg (dry weight), the same as in a regular batch culture. ATP dropped as usual when the stationary phase was reached.

The cells began to recover from the low specific CFU values after 4 or 7 h for plate counts on G2 and N8 agar, respectively (Fig. 3c). The maximum values during the third growth phase were about two-thirds of the values obtained with regular batch cultures. During the stationary phase, the specific CFU values dropped again toward the original low values.

Shift-down experiments. Cells of *C. johnsonae* were injected into a fresh batch culture containing only mineral medium without glucose to simulate immediate starvation. The same cells as in the three shift-up experiments were used in parallel to shift-down experiments and the results are shown in Fig. 4a to c. In these experiments, cells from the fast-growing chemostat cultures (early steady state) showed a strong decrease (70%) in glucose uptake within the first 0.5 h (Fig. 4a). After 8 h, the uptake was less than 8% of the initial value, and 24 h later, it was below the detection limit (data not shown; Höfle, Ph.D. thesis). In contrast, the decrease in the uptake potential was much slower for cells from the slowly growing chemostat cultures (Fig. 4b). After 8 h, the uptake potential was still over 80% of its initial value. Studies with longer starvation periods with these slowly growing cells showed that even after three days over one-third of the original uptake potential was maintained by these cells (Höfle, Ph.D. thesis). The uptake of the long-term-adapted, fast-growing cells decreased very rapidly at the beginning and slowed down after 1 h (Fig. 4c). After 8 h, the uptake potential was only 30% of the initial value and still apparently decreasing. These cells thus showed a decrease in the uptake potential as strong as the short-term-adapted cells but had a higher potential to start with.

The cellular ATP content dropped within the first 0.5 h in all shift-down experiments to values around 0.6 ng of ATP per μg (dry weight) and remained in the range of 0.4 to 0.6 ng of ATP per μg (dry weight) (Fig. 4a to c). This was about one-half of the value for cells from logarithmically growing batch or chemostat cultures and is comparable to values found in the early stationary phase of regular batch cultures (Höfle, Ph.D. thesis).

The specific CFU values on both agars did not change during the 8-h incubation in all shift-down experiments (data not shown). Also, during longer starvation experiments (3 days), no decrease in the viability of cells from any culture was detected (Höfle, Ph.D. thesis).

DISCUSSION

Effect of nutrient shift-up on ATP content. The changes in cellular ATP content were quite similar during all three shift-up experiments. They can be divided into four different phases: (i) a maximum during the first 0.5 h of up to 137% of the initial value, (ii) a transient phase with a minimum of 77%, (iii) a constant high phase (100%) with maximum growth, and (iv) a constant low phase around 50% of the high phase as soon as the stationary phase is reached. The initial maximum can be explained as an overshoot reaction of the energy-producing metabolism in response to the sudden presence of large amounts of substrate (1). This finding indicates that increases in the ATP content of microbial communities shortly after the addition of large amounts of organic substrates, as observed recently by Karl et al. (12), are probably due more to an increase in the ATP content of the biomass than to an increase in the biomass itself. The association of low growth rates with the first transient growth phase (not found in the first shift-up experiment),

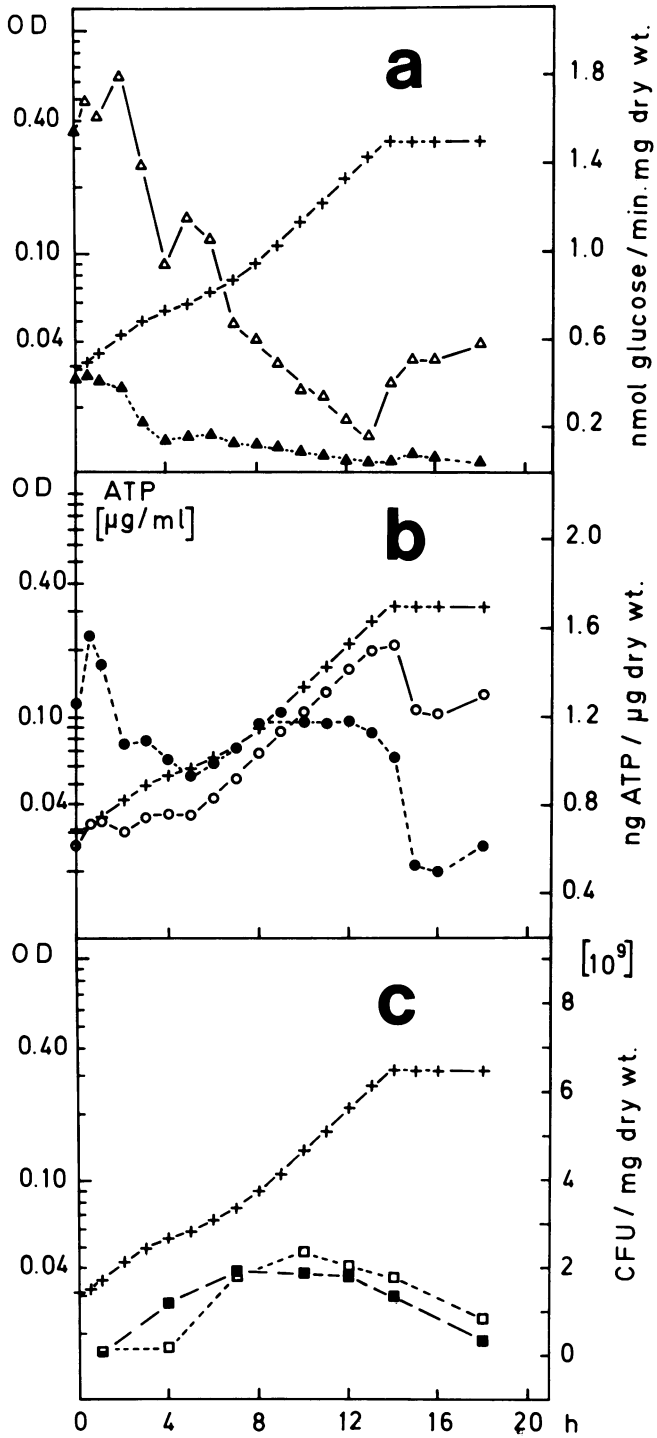

FIG. 3. Third shift-up experiment. *C. johnsonae* cells from a fast growing (D = 0.15 h^{-1}) but long-term-adapted (1,245 h), glucose-limited chemostat culture were injected at 0 h into a fresh batch culture. Graphical arrangement and symbols are as in Fig. 1.

suggests that a certain level of ATP in the cells must be maintained to allow growth at the maximum growth rate. The threshold for maximum exponential growth is 1.1 ng of ATP per µg (dry weight) for *C. johnsonae*. At values below this threshold, growth was limited. This cellular ATP level was also observed over a broad range of growth rates in glucose-limited chemostat cultures (Höfle, Ph.D. thesis). ATP content dropped in chemostat cultures below this level only at growth rates lower than 10% μ_{max}, when signs of metabolic disorder, e.g., leakage of metabolites, occurred (Höfle, Ph.D. thesis). The constant cellular ATP levels that characterized the late logarithmic and stationary phases are also typical of normal maximal logarithmic growth and of phases where, because of the lack of substrate, no growth at all occurs. In general, the consistency of the ATP patterns in all experiments indicates that, despite the different histories and physiological states of the cells, similar regulatory mechanisms may control energy metabolism.

TABLE 1. Growth rates during the different shift-up experiments with glucose-limited cells of *C. johnsonae*

Expt	Time span (h)	Growth rate (h^{-1})[a]	% μ_{max}[b]	% Dilution rate[c]
1	0–10	0.203	102	135
2	0–1	0	0	0
	1–7	0.086	43	287
	7–17	0.165	83	550
3	0–3	0.161	81	107
	3–7	0.103	52	69
	7–13	0.216	109	144

[a] Specific growth rate derived from the semilogarithmic regression of the OD values in the given time span.
[b] Specific growth rate as a percentage of the maximum growth rate measured in regular batch cultures.
[c] Specific growth rate as a percentage of the dilution rate at which the inoculum had been grown.

Effect of nutrient shift-up on uptake and mineralization potentials. Earlier studies of the glucose uptake system of *C. johnsonae* have shown that this bacterium expresses two different glucose uptake systems depending on the ambient substrate concentration (9; Höfle, Ph.D. thesis). At high glucose concentrations (>0.1 mmol/liter), as in a batch culture or a fast-growing chemostat culture, a low-affinity but high-maximum-velocity glucose uptake system operates (K_m = 8.5 µmol/liter, V_{max} > 150 nmol/min per mg [dry weight]). At low substrate concentrations, in slowly growing or long-term-adapted chemostat cultures, a second, high-affinity but low-maximum-velocity uptake system for glucose operates (K_m = 1.6 µmol/liter, V_{max} = 11.2 nmol/min per mg of dry weight).

Only the first shift-up experiment, in which the cells expressed the low-affinity system, showed no large changes in the uptake and mineralization potentials, except a small initial peak. In the two other shift experiments, in which cells with the high-affinity uptake system were used, the uptake and mineralizaiton potentials decreased to less than one-third of the initial rate before the cells grew at their maximum rate. The substrate uptake potential was measured in all assays at a very low substrate concentration (1 µM). At that concentration, the low-affinity system showed a 10-times-lower uptake rate than the high-affinity system (9). The decrease of the apparent glucose uptake potential indicates, therefore, that the cells switched from the high-affinity uptake system (low V_{max}) to the low-affinity uptake system (high V_{max}) during the shift-up experiments. This switch to the low-affinity system was necessary to enable the cells to take up enough substrate for the synthesis of new cell material at high growth rates. Chemostat measurements and calculations showed that above 20% μ_{max}, the maximum velocity of the high-affinity system was not sufficient to

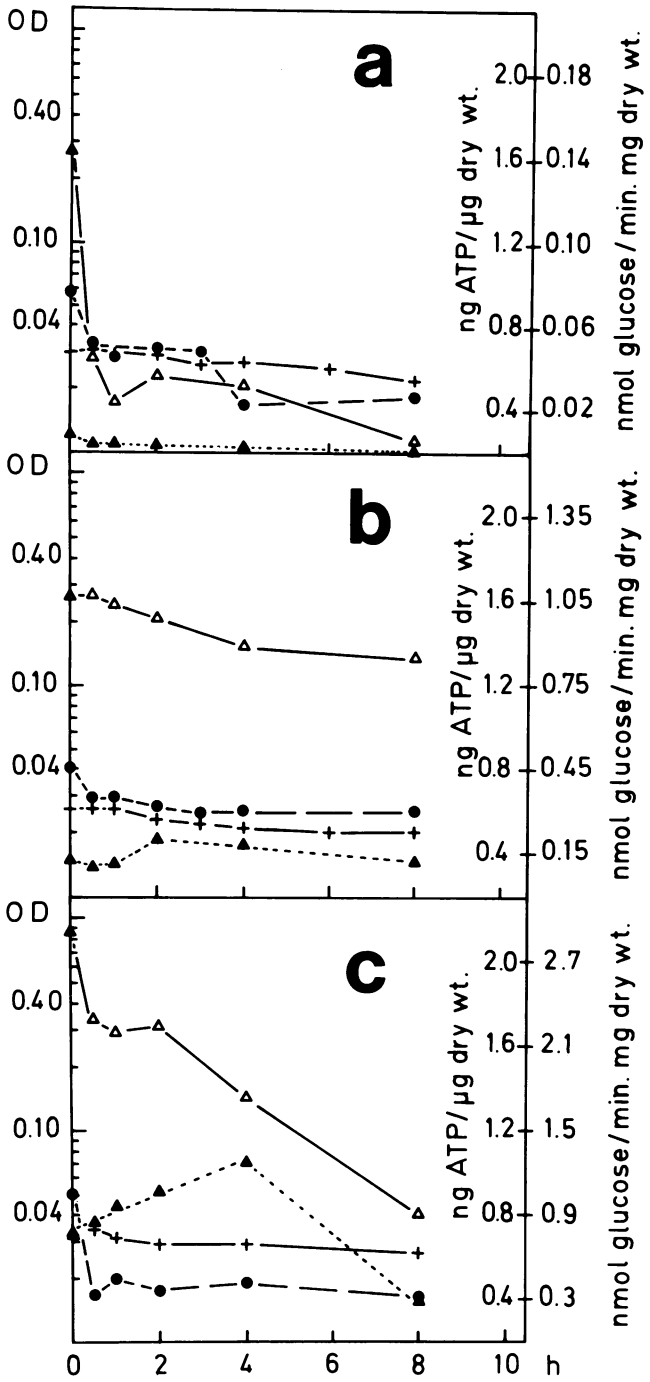

FIG. 4. Shift-down experiments. *C. johnsonae* cells from different chemostat cultures were taken out and incubated under identical conditions. (a) Cells from a fast growing chemostat culture (D = 0.15 h^{-1}). (b) Cells from a slowly growing chemostat culture (D = 0.03 h^{-1}). (c) Cells from a fast-growing, (D = 0.15 h^{-1}) but long-term-adapted (1,245 h) chemostat culture. Symbols are as in Fig. 1.

account for the flux of substrate through the cells (Höfle, Ph.D. thesis). That the substrate uptake potential limits the growth rate can also be seen directly in the second and the third shift-up experiments. In these experiments, the maximum growth rate can only be attained after the uptake potential has dropped significantly. In general, this behavior shows that optimizing the substrate uptake for scavenging small amounts of substrate renders bacteria less flexible to large fluctuations in nutrient concentrations.

The mineralization potential, in general, followed the substrate uptake in all shift-up experiments, indicating the close tie between uptake and metabolization of the substrate. However, the mineralization potential showed a less dramatic and smoother curve, indicating that the regulatory mechanisms of the cellular metabolism dampen some of the strong variations in the substrate uptake potential.

Growth responses after a nutrient shift-up. The growth response to the new substrate concentration was without a significant lag phase in the first shift-up experiment. This fast response to new substrate concentrations renders *C. johnsonae* very flexible at the high-growth-rate range. *Escherichia coli*, for example, showed a transient phase of 1 to 2 generations before it reached its maximum growth rate in a glucose mineral medium in shift-up experiments from glucose-limited chemostat cultures above 60% μ_{max} (7, 14). Shifts of slowly growing or long-term-adapted cells of *C. johnsonae*, however, resulted in transient growth responses and longer adaptation periods (Fig. 2 and 3). *E. coli* had also not shown immediate adaptation to high growth rates from growth rates of lower than 60% μ_{max} (7). Both species reached their maximum growth rate after 2 to 3 generations in transient growth. *C. johnsonae* may therefore be regarded as more flexible than *E. coli* in terms of adaptability to new glucose concentrations.

Cells of *C. johnsonae* growing at 0.03 h^{-1} in a glucose-limited chemostat had an RNA content of 12% (dry weight) in comparison to 20% at their maximum growth rate in a comparable batch culture (Höfle, Ph.D. thesis). Their protein synthesis reserve potential (13), characterized primarily by the RNA content, may therefore not have been high enough for immediate growth at the maximum rate. In contrast, the transient growth kinetics of the long-term-adapted, fast-growing cells cannot be explained by a low-protein-synthesis reserve potential. Their RNA content was not significantly lower than the RNA content of the short-term-adapted cells which showed no significant lag after the shift-up. This transient growth is most easily understood in the context of switching from the high-affinity uptake system, which these cells express during their long-term adaptation (10), to the low affinity system. Therefore, for transient growth responses, not only the protein synthesis reserve potential but also the substrate uptake reserve potential is important if the microorganism has more than one uptake system for the limiting substrate.

Effect of nutrient shift-up on the specific CFU values. Most of the changes of the specific CFU values could be explained by changes in the cell number of biomass ratio. The general trend was that the faster the cells grew, the larger they were, which in turn reduced their CFU values per unit of biomass. This caused a decline in the logarithmic phase of the shift experiments. The only exception to this pattern was the third shift-up experiment. Here, the cells started out with very low specific CFU values (only 15% of the regular batch culture) which was due to an inability to grow on agar plates and not to a low vitality of the cells (10). These cells almost recovered from this inability during the later logarithmic phase, but in the stationary phase, most of the cells (60 to 78%) lost this ability again. This indicates that the cells have not completely changed back to normal and still contain a memory of their history 2 to 3 generations earlier. This memory effect can also be seen in the uptake potentials of the second and the third shift-up experiment (Fig. 2a and 3a),

which do not decrease as in a regular batch culture when the stationary phase is reached.

Responses to starvation (shift-down). The shift-down experiments, performed with cells from different growth conditions, clearly showed that the response of the uptake and mineralization potentials of these cells to starvation differs strongly. Slowly growing cells are able to maintain their high uptake and mineralization potential for a much longer time and at a much higher level than fast-growing cells. The slowly growing cells are therefore much better prepared for times of starvation. They are able to compete more efficiently for new substrate after a starvation period because their uptake potential is still high. The decreasing cellular ATP content does not directly affect the uptake potential in the slowly growing cells. Therefore, it can be assumed that the energy supply for the high-affinity uptake system is not coupled directly to ATP, which is supported by inhibitor studies (10). The mineralization rate increased during the first 2 to 4 h relative to the uptake rate in the slowly growing and the long-term-adapted cells (Fig. 4b and c). This could indicate that the cells shift their metabolism away from assimilation and focus it on energy production. A similar response was observed recently with a marine *Pseudomonas* sp., but on a much longer time scale (16). In general, these starvation adaptations of *C. johnsonae* may be selectively advantageous during short-term fluctuations of nutrients, such as during diel cycles, observable in the euphotic zone of most aquatic environments (6, 20, 21).

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