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Changes in the Oxygen Uptake by *Staphylococcus aureus* Oxford in Response to Changes in the Environment

Zmiany w poborze tlenu przez *Staphylococcus aureus* Oxford spowodowane zmianami warunków hodowli

Изменения в поглощении кислорода *Staphylococcus aureus* Oxford вызванные изменениями в условиях культуры

It is a known fact that the carbohydrate breakdown pattern of bacteria can undergo changes in response to changes in the environment. Under normal conditions *S. aureus* metabolizes glucose by glycolysis and subsequent oxidation of pyruvic acid in TCA cycle (tricarboxylic acid cycle), although the link between those two pathways is rather weak (3, 6, 15, 16). The existence of the pentose cycle in the glucose breakdown in staphylococci has also been reported (2, 4, 7, 16). No Entner-Doudoroff pathway was found (16).

The aim of this paper was to study the effect of glucose and anaerobiosis on the oxidation of some carbohydrates and amino acids by *S. aureus* Oxford.

MATERIALS AND METHODS

Strains

S. aureus Oxford was used in all experiments reported here.

Cultures

S. aureus Oxford was maintained in a dry state and subcultured for each experiment. Nutrient broth and plain agar were used throughout experiments.

Exponential growth of cells

Dry cells were suspended in 10 ml of broth, then incubated for 18 hrs at 37°. Next day 100 ml of broth was inoculated with overnight broth culture and incubated for 5 hrs at 37° on a shaker. When the optical density was equivalent to 1 mg/ml of dry cells, the culture was cooled down and maintained overnight at 4°. The following day the culture was warmed up, diluted with the same volume

of fresh broth and incubation continued for 3 hrs on a shaker at 37°. The bacteria were washed three times with saline by centrifugation. In some experiments glucose was included into the medium.

Stationary phase culture

Dry cells were incubated overnight in broth, then the culture was transferred on agar (in Roux bottles) and incubated for 21 hrs at 37°. The bacteria were washed off with saline and then kept in saline at 4° for 24 hrs to reduce endogenes respiration. To some cultures glucose was added.

Anaerobic growth

10 ml of overnight broth culture of *S. aureus* Oxford was added to 500 ml of broth and incubation continued in completely filled glass-stoppered bottles at 37° for 18 hrs. The organisms were then harvested and washed with saline by centrifugation. Some cultures contained glucose.

Manometric experiments (19).

1 ml of cell suspension (4—5 mg of dry weight), grown under various conditions, was added to the main Warburg vessel, then 1 ml of 0.1 M phosphate buffer, pH 7.0. To the sidearms 0.5 ml of 0.5 M substrated were added: glucose, ribose, succinate, pyruvate, citrate, fumarate, acetate, lactate, gluconate, alanine, glycine, glutamate and serine. To the central well 0.2 ml of 20% KOH was added. After 20 min. Incubation substrates were transferred from the sidearms to the main vessels and incubation continued for 60 min. Readings were taken every 10 min. To determine the quotiens QO_2 suitable portions of the suspensions were dried (10 hrs, 110°) and weighed.

RESULTS

As can be seen from table 1, lactate is the best substrate for respiration of *S. aureus* Oxford. These data are in agreement with previous results of one of us (18). To a lesser extent — pyruvate, glucose and ribose were oxidized. Gluconate, fumarate, succinate and acetate were rather poor substrates both for exponential cells and stationary ones.

It was found that the effect of glucose incorporated into growth medium on the ability of *S. aureus* Oxford to oxidize above mentioned substrates is concentration dependent. 0.2% of this sugar had only a slight effect on the oxidation of the majority of substrates, only in the presence of pyruvate and acetate a considerable reduction in the oxygen uptake was noticed. Cells grown on higher concentration of glucose (1%) did not respire in the presence of all substrates used, except glucose and lactate. The oxidation of these two substrates was only partially reduced — inhibition being 55.0% and 35.5% respectively. It is noteworthy that the endogenous respiration was also completely suppressed by the addition of 1% glucose to the growth medium.

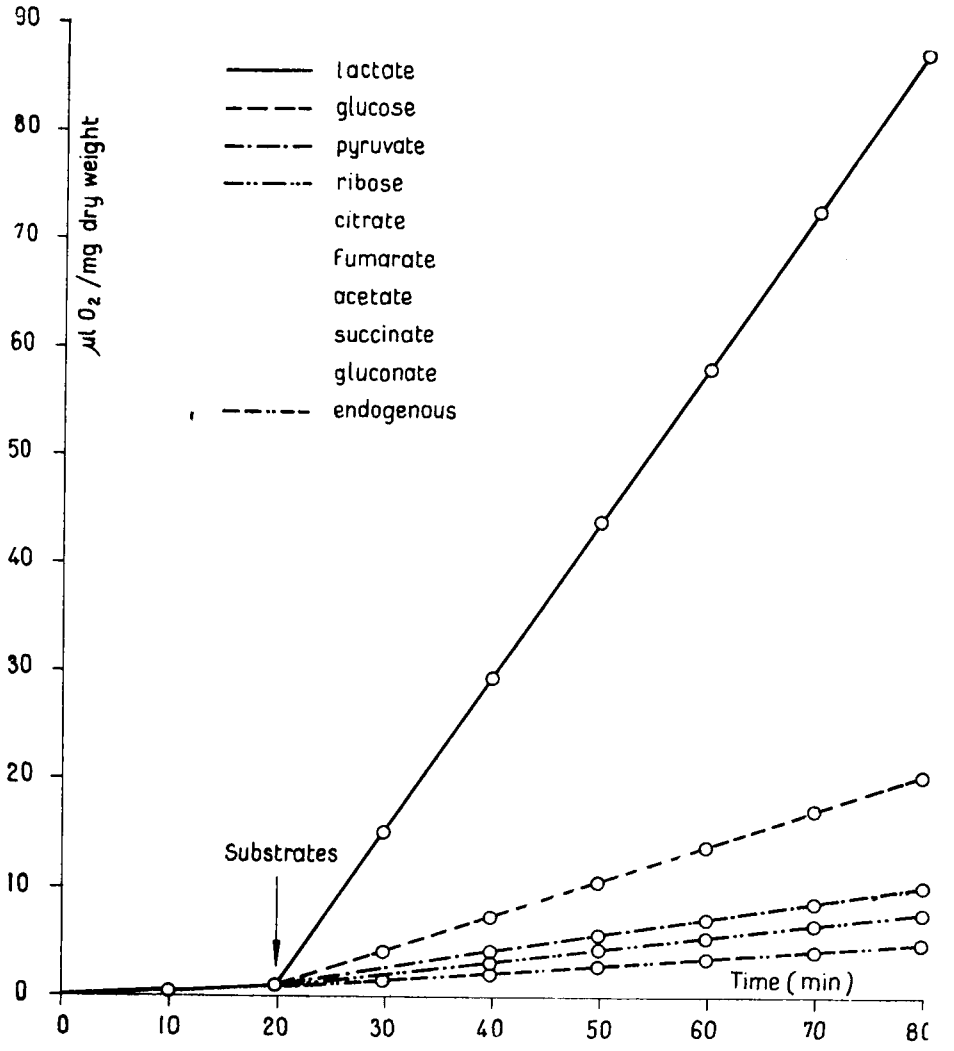
Fig. 1. presents the oxidation of various substrates by cells grown under anaerobic conditions. It is evident that the lack of oxygen during growth of *S. aureus* Oxford had similar effect as glucose on its subse-

Table 1. The effect of glucose on the oxidation of some substrates by *S. aureus* Oxford

Wpływ glukozy na utlenianie niektórych substratów przez *Staphylococcus aureus* Oxford

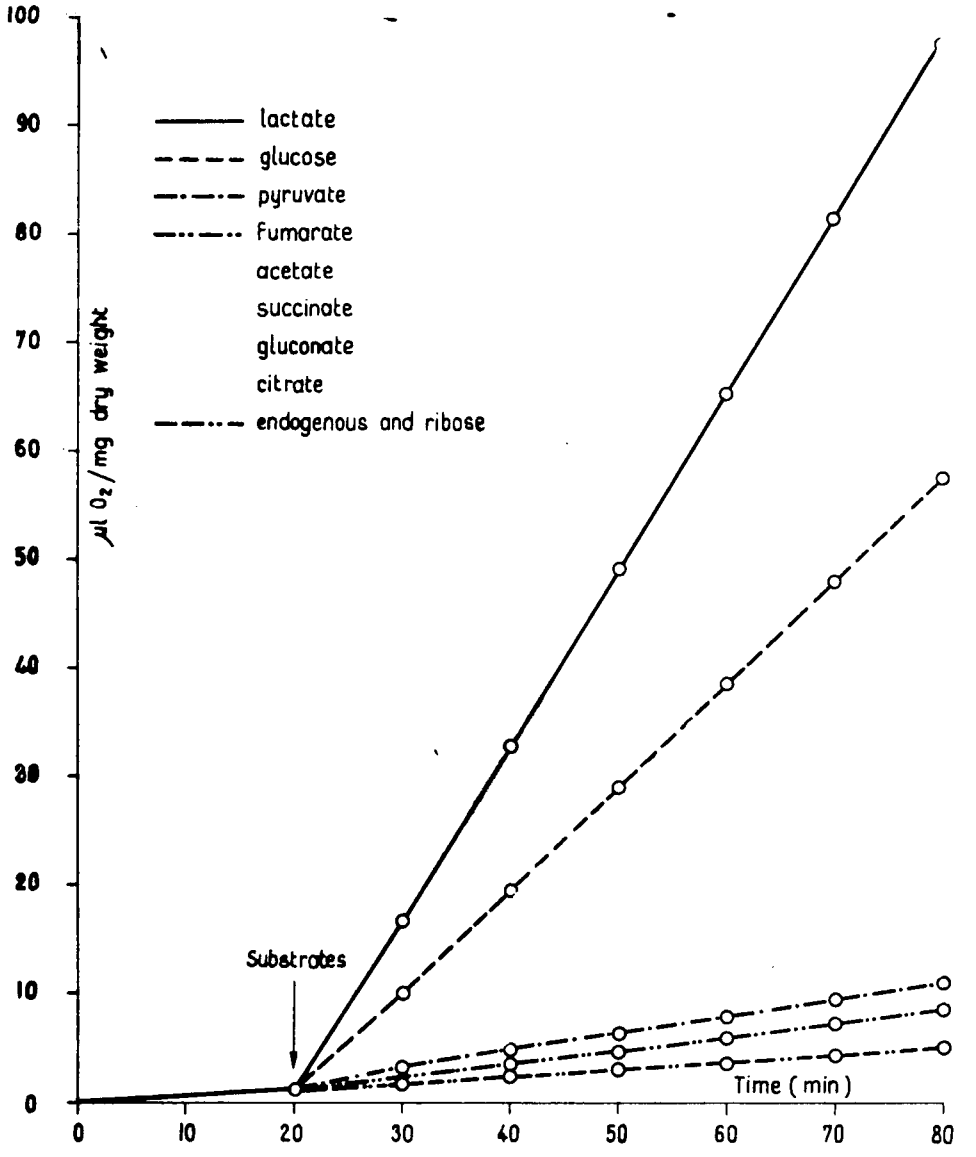
Type of cells	µl O_2/hr/mg dry weight (endogenous subtracted)									
	Endogenous	Glucose	Ribose	Citrate	Pyruvate	Fumarate	Lactate	Acetate	Succinate	Gluconate
Exponential phase cells	15.04	80.01	54.17	—	95.35	10.32	233.84	20.11	11.35	6.31
Exponential phase cells + 0.5% glucose	1.05 (93.1)*	38.76 (51.6)	— (100.0)	—	12.39 (87.1)	1.04 (90.0)	170.08 (27.3)	— (100.0)	1.49 (87.0)	2.28 (63.9)
Stationary phase cells	7.36	93.55	71.24	4.62	95.22	30.02	215.65	24.98	28.50	8.64
Stationary phase cells + 0.2% glucose	6.58 (10.6)	83.59 (10.7)	60.12 (15.7)	— (100.0)	58.18 (39.1)	22.93 (23.7)	208.16 (3.5)	13.26 (47.0)	22.61 (20.7)	7.58 (12.3)
Stationary phase cells + 0.5% glucose	3.11 (57.8)	59.32 (36.6)	2.98 (95.9)	— (100.0)	21.91 (77.0)	1.35 (95.6)	158.84 (26.4)	3.07 (87.7)	1.62 (94.4)	2.56 (70.4)
Stationary phase cells + 1.0% glucose	— (100.0)	42.1 (55.0)	— (100.0)	— (100.0)	— (100.0)	— (100.0)	139.2 (35.5)	— (100.0)	— (100.0)	— (100.0)

* Figures in brackets — % inhibition of oxygen uptake.



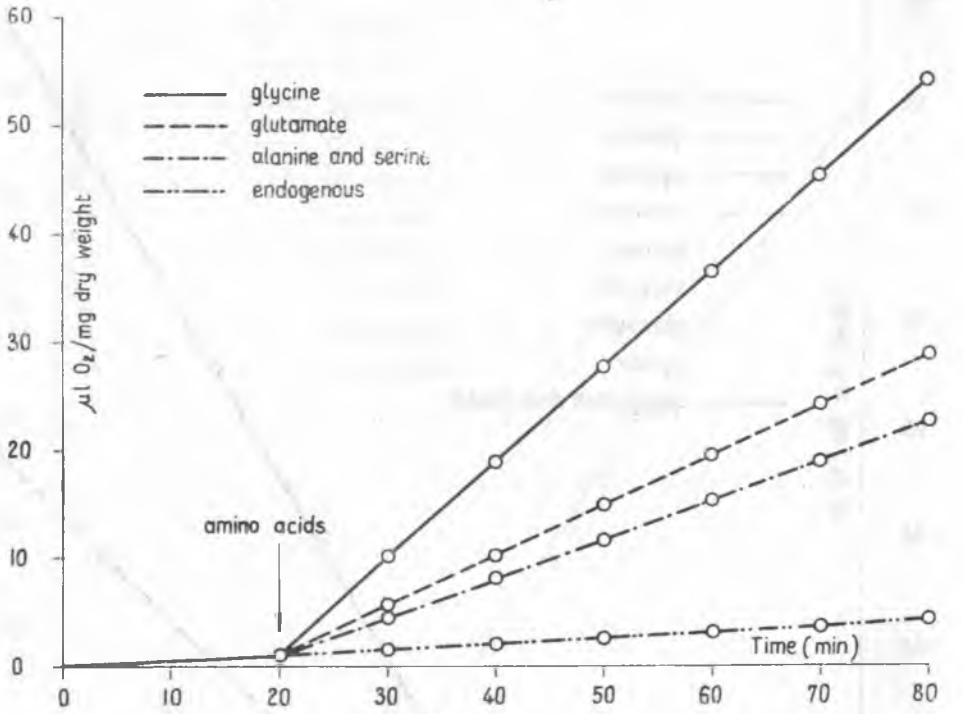
Ryc. 1. Utlenianie niektórych substratów przez komórki *Staphylococcus aureus* namnażane w warunkach beztlenowych
Oxidation of various substrates by *S. aureus* Oxford grown anaeriobically

quent ability to oxidize added substrates. None of the compounds, except lactate and to a very slight degree glucose, stimulated respiration of the organism tested. Besides, the amounts of oxygen consumed by anaerobically grown cells in the presence of lactate and glucose were much lower than by cells grown in the presence of oxygen (table 1). (Fig. 1.). The addition of 0.5% glucose to anaerobically grown culture enhanced only the rate of glucose oxidation while the rate of oxygen uptake in



Ryc. 2. Utlenianie niektórych substratów przez komórki *Staphylococcus aureus* hodowane w warunkach beztlenowych w obecności 0,5% glukozy
Oxidation of various substrates by *S. aureus* Oxford grown anaeriobically in the presence of 0.5% glucose

the presence of other substrates remained the same. (Fig. 2). Fig. 3. presents the ability of *S. aureus* Oxford resting cells to use amino acids as substrates for respiration. As can be seen, „starved" cells, that is those in



Ryc. 3. Utlenianie niektórych aminokwasów przez głodzone komórki *Staphylococcus aureus* Oxford

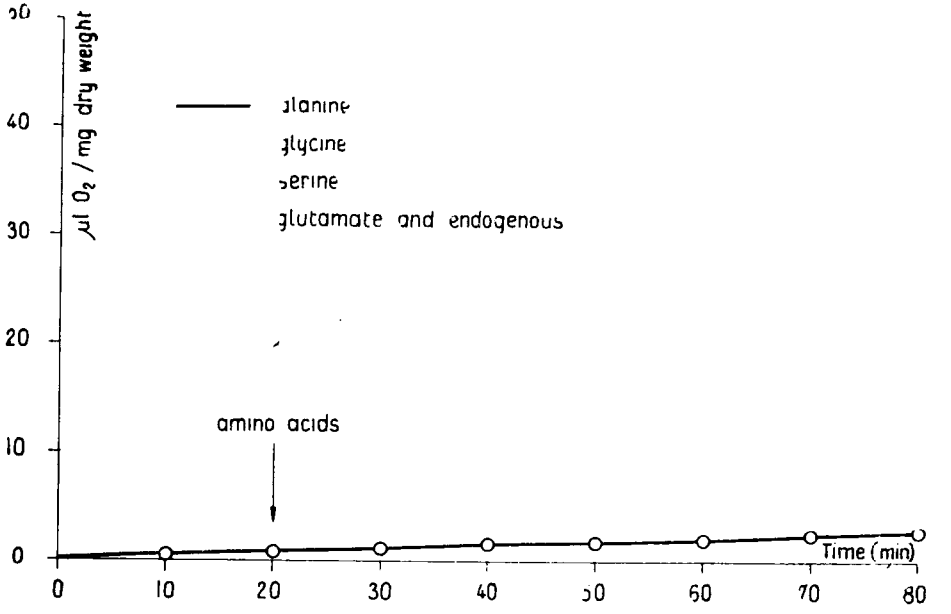
Oxidation of some amino acids by resting "starved" cells of *S. aureus* Oxford

which endogenous respiration was highly reduced, oxidized amino acids, but to a different degree. Most readily were oxidized: glycine, then glutamate, alanine and serine. (Fig. 3). It is interesting to see that „starved" cells, grown in the presence of 0.5% glucose, did not use amino acids for respiration at all. (Fig. 4).

DISSCUSSION

There are many examples in literature of the repression of enzyme synthesis by glucose. In particular, the presence of glucose can prevent the formation of inducible enzymes necessary for the utilization of other sugars (11, 12, 13). The results presented here indicate that glucose can also prevent the formation of enzymes necessary for the complete oxidation of carbohydrates, via TCA cycle since cells grown on glucose could not oxidize those substrates which are degraded in this cycle: acetate, pyruvate, citrate, fumarate and succinate. Also the hexosemonophosphate pathway substrates — ribose and gluconate — were not oxidized by those cells. It has been noticed that this effect of glucose was concen-

tration dependent. According to Collins and Lascelles (1) and Strasters and Winkler (16), the inability of glucose grown staphylococci to oxidize intermediates of TCA cycle was correlated with the diminished activity of enzymes of this cycle in cell-free system. In our experiments, cells grown on 1% glucose could only oxidize glucose and lactate, but to a lesser degree than cells grown without it, though, as was pointed out by Strasters and Winkler (16), the activity of some enzymts of the glycolytic route in cells grown on glucose was higher than in cells grown in its absence. The lower rate of oxidation of glucose and lactate by glucose grown cells might be due to the suppression of the subsequent oxidation of breakdown products of these two substrates via TCA cycle.



Ryc. 4. Utlenianie niektórych aminokwasów przez głodzone komórki *Staphylococcus aureus*, hodowane w obecności 0,5% glukozy

Oxidation of some amino acids by resting "starved" cells of *S. aureus* Oxford grown in the presence of 0.5% glucose

A similar effect was observed when cells were grown anaerobically; except lactate and glucose neither of the above mentioned substrates was oxidized. It is thus evident that the ability to oxidize TCA cycle intermediates is controlled by two factors — catabolite repression and oxygen; however the two effects are not mutually exclusive. As has been shown, oxygen could not enhance the synthesis of enzymes necessary for oxidation of TCA cycle intermediates in aerobic system when the organism was growing at the expense of glucose.

It has been established that amino acids of the pool serve as substrates for endogenous respiration of staphylococci (8, 9, 10, 14, 17, 18). In our experiments „starved" cells used amino acids for respiration, while cells grown on glucose did not possess that ability. It is probable that staphylococci grown on complex media in the absence of glucose, can satisfy the energy requirements by oxidation of amino acids by mechanism which may involve the TCA cycle. Addition of glucose suppresses the synthesis of TCA cycle enzymes, rendering the cell unable to oxidize amino acids. Probably in the presence of glucose sufficient energy for growth is gained by incomplete oxidation of this sugar to the acetate stage (5), and the operation of the TCA cycle is not necessary (1). It is thus clear that in cells grown on glucose the endogenous respiration was also suppressed, since, as was already mentioned, amino acids of the pool were the substrates for it.

According to Magasanik et al. (11) and Neidhardt and Magasanik (13), glucose acts as a repressor being more rapidly metabolized than other carbon sources, so that under growing conditions intermediary metabolites accumulate which repress the inducible enzyme synthesis concerned with the utilization of alternative energy sources.

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STRESZCZENIE

Założeniem pracy było zbadanie wpływu glukozy na zdolność *Staphylococcus aureus* Oxford do utleniania niektórych węglowodanów i aminokwasów. Wykazano, że dodatek glukozy do podłoża w stężeniu 1% hamuje całkowicie utlenianie pirogronianu, octanu, fumaranu, bursztynianu, cytrynianu, rybozy i glukonianiu; jedynie utlenianie glukozy i mleczanu było tylko częściowo zahamowane. Efekt glukozy nasilał się wraz ze wzrostem stężenia tego cukru w podłożu wzrostowym.

Podobny efekt hamujący wywoływało namnażanie badanego szczepu w warunkach beztlenowych.

Głodzone komórki *S. aureus* Oxford zużytkowywały aminokwasy: kwas glutaminowy, alaninę, glicynę i serynę jako substraty oddychania, podczas gdy identycznie namnażane komórki, ale w obecności 0.5% glukozy, nie posiadały tych zdolności. Endogenne oddychanie, dla którego substratem są aminokwasy puli, było również hamowane przez dodatek glukozy do podłoża wzrostowego.

Z przeprowadzonych doświadczeń wynika, że *S. aureus* Oxford namnażany w obecności glukozy czerpie energię do wzrostu z glikolizy, mając nieaktywny cykl kwasów trójkarboksylowych.

РЕЗЮМЕ

Целью работы было исследование влияния глюкозы на способность *Staphylococcus aureus* Oxford окислять некоторые углеводы и аминокислоты. Обнаружено, что добавление глюкозы к среде с концентрацией 1% полностью ингибирует окисление пирувата, ацетата, фумарата, сукцината, цитрата, рибозы и глюконата; окисление глюкозы и глюконата тормозилось только частично. Влияние глюкозы усиливалось вместе с повышением концентрации этого сахара в ростовой среде. Аналогичный ингибирующий эффект вызывало выращивание исследованного штамма в анаэробных условиях.

Голодающие клетки *S. aureus* Oxford использовались в качестве субстратов для дыхания следующими аминокислотами: аланином, глици-

ном, серином и глютаминовой кислотой. Эти же клетки, но выращенные в присутствии 2,5%, не использовали вышеупомянутых аминокислот.

Эндогенное дыхание за счет внутриклеточных аминокислот ингибировалось также добавлением глюкозы в ростовой среде.

Из проведенных исследований следует, что *S. aureus* Oxford выращиваемый в присутствии глюкозы, черпет энергию для роста из гликолиза, обладая неактивным циклом трикарбоновых кислот.