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BIOCHEMICAL STUDIES IN CONNECTION WITH THE VIRULENCE OF CERTAIN
SUPPURATING AGENTS AND QUANTITATIVE USE OF BLOOD INJECTIONS
(FIRST REPORT: THE OXYGEN CONSUMPTION OF
PASTEURELLA ANTIQUICIDA)

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The problem of the connection between the metabolism of microorganisms and their pathogenicity is difficult to resolve because of its complex nature. A thoroughgoing explanation of an illness requires a characterization not only of the pathogenic causative agent but also of the host organism. The problem is also complicated by the insignificant knowledge that we possess on the biochemical ties between the potential of the infectious causative agent and the host organism during the process of infection.

There are data in the literature on differences in the biochemical activity of virulent and avirulent strains of given bacterial varieties, e.g., Staphylococcus (Literature references 9, 10). Virulent strains of Mycobacterium possess significantly lower respiratory capability than avirulent strains (Literature reference 11). It has been established with Br. abortus and Br. suis that strains with lower virulence oxidize glutamine to a much greater extent than strains which are powerfully virulent. Studies of intact cells of Past. tularensis show that the oxidizing capacity of the virulent strains is greater than that of strains which are weakly virulent (Literature

reference 13). A correlation between catalatic activity and virulence has been established with M. tuberculosis, Brucella, and Past. pestis. Strains with low virulence manifest weak catalatic activity, virulent strains high catalatic activity (Literature reference 16). These studies in vitro represent a contribution which is not sufficient to explain the complex connection in vivo.

Interesting data have been established by Al. Toshkov and associates in connection with the postmortem multiplication of a number of septicemic agents and causative agents of blood infections (Literature references 2, 3, 4, 5, 6, 7, 14). In accordance with postmortem multiplication in the cadavers of animals, a sharp increase was noted in virulence, which can rise to improbable heights (e.g., 10^{-30} to 10^{-40} in the case of Past. avicida). This phenomenon has been demonstrated with Past. pseudotuberculosis, Erys. rhusiopathiae, Bact. pyocyaneum, streptococci, staphylococci, List. monocytogenes, and Salmonella typhimurium and is currently the object of extensive study.

In the present article, we are reporting the initial results of biochemical studies of Pseudomonas aeruginosa in connection with its heightened virulence in accordance with postmortem multiplication in cadavers or in cultivation in defibrinized blood under conditions described in the experimental section.

Materials and Methods

About bacterial cultures: we used suspensions prepared from 24-hour cultures of Pseudomonas aeruginosa on ordinary agar.

The term "initial strain" below refers to a strain used to infect guinea pigs and as an inoculum in cultivation in defibrinized blood.

The term "virulent strain" below refers to strains with heightened virulence in the cadavers of guinea pigs dead following experimental infection with Ps. aeruginosa and to strains with heightened virulence following cultivation in defibrinized blood.

Substrata: 0.1 M solutions of glutamate, glycerol, acetate, glucose, lactate, pyruvate, ethanol, citrate, fumarate, maleinate, and succinate. Sodium salts of acids were used.

A polarigraphic method with a dripping mercury electrode was used to determine the speed of oxygen consumption (Literature reference 1).

Results and Discussion

The oxidizing capacity of [illegible] cells of Ps. aeruginosa in terms of the various substrata [illegible] always determined in combination with their endogenous respiration. By convidence, the initial strain and the virulent strain [illegible] endogenous respiratory quantity, a comparison of [illegible] respiratory capability under different conditions. In all experiments, bacterial suspensions of the two strains with equal density were used.

The oxidizing breakdown of the substrata by the intact cells of the initial and virulent strains proceeded at varying speed. The oxygen consumption* of the virulent strain is greater than that of the initial strain of Ps. aeruginosa. The results of our experiments are shown in graphic form in Figure 1.

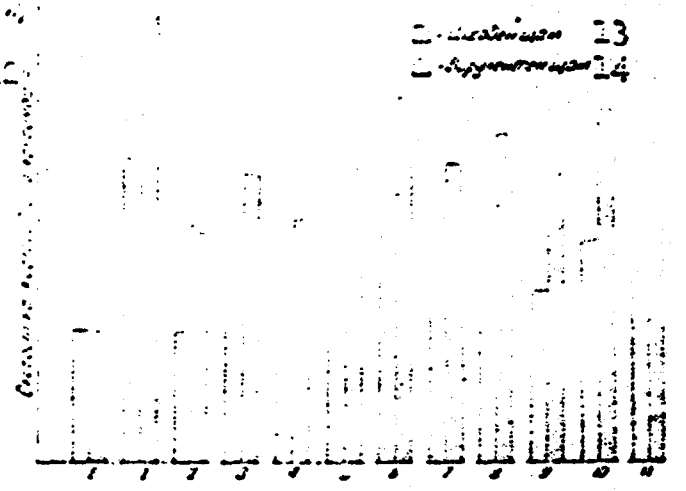

Figure 1. Substrata of respiration.

1) Endogenous respiration; 2) Glutamate; 3) Glycerol; 4) Acetate; 5) Lactate; 6) Pyruvate; 7) Ethanol; 8) Citrate; 9) Formate; 10) Maleinate; 11) Succinate; 12) Speed of oxygen consumption; 13) Initial strain; 14) Virulent strain.

If we compare the values for each substratum, we can establish that the virulent strain possesses roughly 50 percent more active respiration than the initial strain. In all probability, the difference observed reflects a differing need for energy in the two strains.

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* We term "oxygen consumption" in this text refers to the speed at which oxygen is consumed.

We consider the ability to oxidize glucose and glycerol under aerobic conditions to be specific solely to the virulent strain. This is a notable aspect of our studies. Detailed study was given to this characteristic feature distinguishing between the two strains.

We should note that the differences observed in oxygen consumption between the initial and virulent strains are the same, regardless of whether the virulent strain of Ps. aeruginosa is isolated from the cadaver of a guinea pig or after cultivation in defibrinized blood. One of the possible ways to explain this established fact is as follows. Ps. aeruginosa produces substantial quantities of hemolysin, which breaks down the erythrocytes in the blood (in the cadaver or defibrinized blood). Under these circumstances, the multiplying bacterial cells possess an adequate concentration of iron ions and structures necessary to the build-up of their cytochrome system. The preliminary studies of this link in the respiratory chain are discouraging. For this purpose, we investigated the sensitivity of the bacterial cells to potassium cyanide, which inhibits the electronic process. The results are shown in Figure 2.

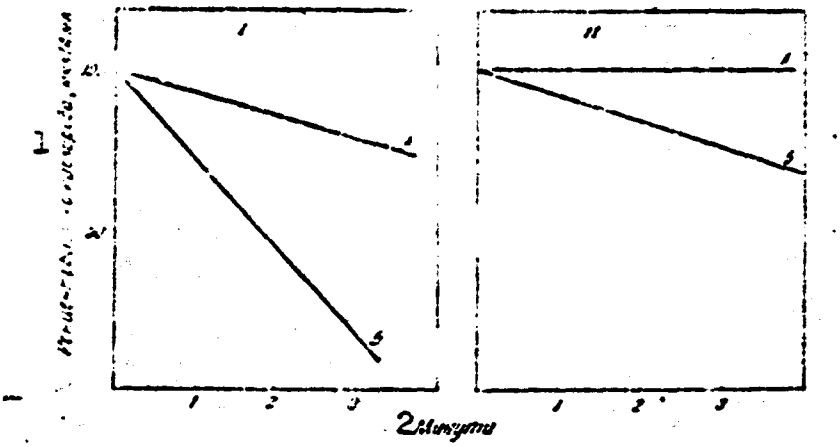

Figure 2.

I) Virulent strain; II) Initial strain; A) Inhibited tests with 10^{-2} of KCN; B) Control tests (substratum of 10^{-1} lactate; pH 7.00; 0.066 M of phosphate buffer); 1) Concentration of oxygen in mkl/4 ml; 2) Minutes.

The experimental data show a certain difference in the oxidizing capacity of the virulent strain and that of the initial strain, which is entirely inhibited under these circumstances. We are inclined to grant the existence of a correlation between the substantial oxygen consumption and resistance of the virulent strain to potassium cyanide, i.e., the greater oxidizing capacity may be determined by the more active cytochrome system. A relationship between the cell inhibitors and that difference cannot be excluded.

Our studies of the dehydrogenase activity of the two strains are another indirect proof. There were no differences between them in the anaerobic dehydrogenation of the substrates used. The elimination of the dehydrogenase element impelled us to investigate the succeeding stages of biological oxidation.

While there is a linear dependence between respiratory activity and the composition of the cytochromes in precipitates (Literature references 13), such a correlation is not always observable with bacteria (Literature reference 6). Yet low respiratory activity and low cytochrome content were established with some mutants of E. coli and Staph. aureus (Literature references 12). Similar investigations should be made in the course of our studies.

In conclusion, we may note in a very general way that the increase in the virulence of Ps. aeruginosa in the cadavers of guinea pigs and after cultivation in defibrinated blood is connected with the heightened biochemical activity of the intact cells, which consume more significant amounts of oxygen. The explication of this complex connection, as well as the inclusion of glucose and glycerol in respiration, will be the object of subsequent investigation.

Conclusions

On the basis of these studies, the following conclusions can be drawn:

1. The speed of oxygen consumption by initial and virulent strains of Ps. aeruginosa differs with the use of 11 substrata. The virulent strain possesses roughly 50 percent more active respiration than the initial strain.

2. The intact cells of the virulent strain are capable of oxidizing glucose and glycerol, which are not the substratum for respiration for the initial strain.

3. The virulent strain demonstrates a certain resistance to the inhibitive effect of potassium cyanide, which entirely inhibits the respiration of the initial strain under the same conditions.(.)

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