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DEPARTMENT OF THE ARMY
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Frederick, Maryland

ON SOME ELECTRONIC ASPECTS OF THE ACTION OF CERTAIN METALLIC CATIONS ON THE GROWTH OF AEROBIC BACTERIA

(ESCHERICHIA COLI) AND OF ANAEROBIC BACTERIA (CLOSTRIDIUM SPOROGENES)

/Following is a translation of an article by Michel Faguet and Mme Andree Goudot of the Pasteur Institute, presented at the 6 July 1961 meeting of the Societe Francaise de Microbiologie (French Microbiology Society) and published in the French-language periodical Annales de l'Institut Pasteur (Annals of the Pasteur Institute), Vol 101, No 6, 1961, pages 860-868./

The important part played by certain metals, principally the transition metals, in oxidation or reduction catalysis and in the inactivation of certain enzymes led us to study and compare their actions on the growth of aerobic germs and anaerobic germs, which differ notably in the mechanism of their oxidations.

In this article we have studied the action of Fe^{2+}, Mn^{2+}, Co^{2+} and Ni^{2+} on the growth of cultures of different aerobic (E. coli) and anaerobic (Cl. sporogenes) germs, and we have attempted to interpret their action by examining the electronic aspect of certain reactions.

TECHNIQUE

a. For the study of aerobic germs we used four strains of Escherichia coli (EM1, EM2, EM12, EC3), cultivated on slant peptone agar kept for eighteen hours at 37° C. and then preserved at +4° C. The nutrient medium, with glucose added at

the rate of 0.3%, was composed, per 1,000 cm^3 of twice-distilled water, of 13.6 g of PO_4KH_2, 0.5 g of KCl, 0.7 g of $SO_4(NH_4)_2$, 0.05 g of $SO_4Mg\ 7\ H_2O$; the pH was adjusted to 7.4.

b. For the study of anaerobic growth, we used strain No. 6053 of the Pasteur Institute collection of Cl. sporogenes, cultivated in a Prevot tube. The medium was composed of a meat broth (peptic digestion), with 1.5% peptone, 0.3% glucose and the pH adjusted to 7.4.

c. The various metals used were utilized in a sulfate state with $7H_2O$, dissolved in twice-distilled water. The final concentrations were of the order of 0.5×10^{-5} mol in a synthetic medium (aerobe), and 0.8×10^{-3} mol in a complex medium (anaerobe).

d. Our studies on growth were made with the aid of our electronic microbiophotometer (Faguet [1] <bracketed numerals refer to similarly numbered items in the bibliography appended at the end>, which gave automatically the microbian multiplication curves. An electromagnetic system allows the agitation of the cultures to be regulated and, in this way, their oxygenation.

e. We calculated the action of the various cations in the exponential part of the growth phase where the relation $y = y_0 \cdot 2^n$ is verified; y represents the density of the culture that grew exponentially on the basis of density y_0, and n is the number of generations. During this phase, no limiting factor comes into play and the growth rate is subjected only to the initial conditions of the medium (Monod [4]).

In all our experiments, we started with an amount of germs close to 5×10^6 per cm^3 of medium.

RESULTS

1. In an initial series on a synthetic medium with E. coli, we studied to determine if, as our previously conducted studies of theoretical chemistry had predicted (Goudot and Faguet [3]), Mn^{2+} acted in a synthetic medium the same way as Fe^{2+}. The analysis of the growth curves showed us that Mn^{2+} can replace Fe^{2+} for the culture of the E. coli that we studied (Figs. 1, 2, 3).

A study of the curves in Figure 1 (E. coli M1) shows that, in culture 1 (Fe^{2+}), the number of divisions per hour (exponential phase) is 0.86; it is 0.84 with Mn^{2+}; 0.30 with Co^{2+} and 0 with Ni^{2+}. With E. coli K2 (Fig. 2), the number of divisions in the same phase is 1 for Fe^{2+} and Mn^{2+}, 0.46 for Co^{2+} and 0 for Ni^{2+}. We observed that Mn^{2+} is at times slightly less active than Fe^{2+}. Moreover, as we see from our curves, a decrease in the oxygenation of the medium produces a decrease in growth.

The presence of Co^{2+} slows it down considerably and Ni^{2+} inhibits it completely, even in the presence of a normal supply of oxygen.

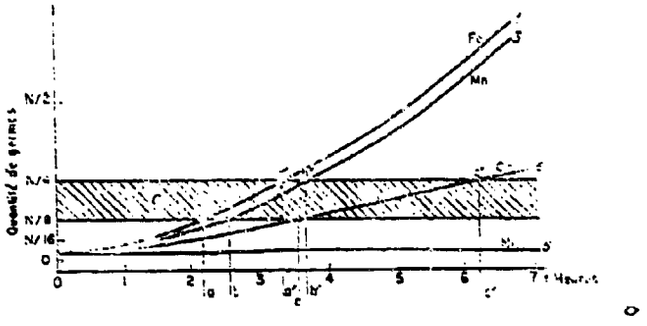

Fig. 1. The four graphs, 1, 3, 5 and 6, represent the growth curves of E. coli M1, in an aerated synthetic medium containing glucose. Each culture contains, respectively Fe, Mn, Co, Ni in the form of their sulfate with seven molecules of water, in a concentration of 0.5×10^{-5} mol. We calculated the speed of growth of each one of the cultures in the interval included between 5×10^{8} germs per cm^3 and 10^9 germs per cm^3. The number of germs doubles for Fe and Mn in 69 minutes; for Co in 158 minutes, and for Ni, growth is zero. If the speed of growth in the presence of Fe and Mn is equal to unity, it is 0.43 for Co and 0 for Ni.

/Legend:/ Quantite de germes = Number of germs (vertical coordinate); Heures = Hours (horizontal coordinate).

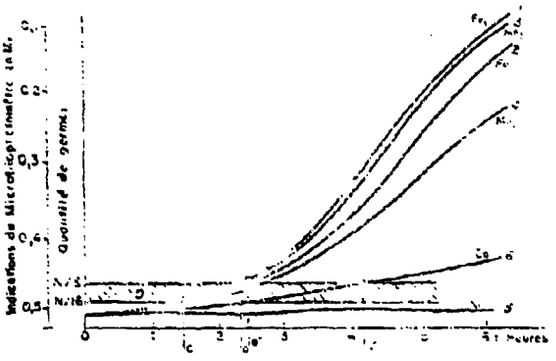

Fig. 2. Growth curves 1, 2, 3, 4, 5, 6 of E. coli K2, in a synthetic medium with glucose and in the presence, respectively, of Fe, Mn, Ni and Co in a concentration of 0.5×10^{-5} mol. In exponential area D, we calculated the growth speed of the differnt cultures: in the presence of Fe (Curve 1) or of Mn (Curve 3), the number of germs of each culture doubles in 60 minutes; in the presence of Co, it doubles in 112 minutes and the culture does not develop in the presence of Ni. If v = 1 for Fe or Mn, it is 0.53 for Co and 0 for Ni. The curves marked Fe_2 and Mn_2 represent growth in the presence of these metals in the same concentration, but without aeration.

/Legend:/ Indications du Microbiophotometre en Mv = Readings of the microbiophotometer in mv (vertical scale); vertical and horizontal coordinates as in Figure 1.

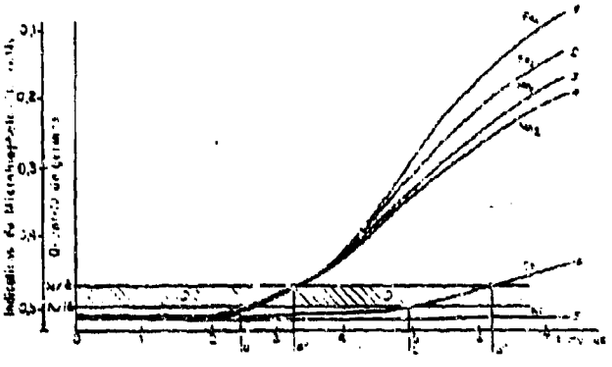

Fig 3. (Caption and legend on following page)

Fig. 3. Growth curve of E. coli C36 in a synthetic medium with glucose. 1 and 3 represent the growths in the presence, respectively, of Fe and Mn in an aerated medium; 2 and 4, in a non-aerated medium, and 5 and 6 translate the growth in the presence of Ni and of Co in an aerated medium (the Fe, Mn, Co and Ni concentration always is 0.5 X 10-5 mol).

/Legend:/ Same scale as in Figure 2 and same coordinates as in Figures 1 and 2.

2. We studied the action of Ni^{2+} and Co^{2+} on cultures of an anaerobic germ; we used a strain of Cl. sporogenes cultivated in special dishes (Faguet /2/) placed in the electronic microbiophotometer adjusted to 37°C. Each dish contained 22 cm^3 of a complex medium, composed of a meat broth (peptic digestion) containing 1.5% peptone, 0.3% glucose and with the pH adjusted to 7.4.

A study of the growth curves (Fig. 4) showed us that the multiplication of this germ was slightly increased by the presence of Ni^{2+} and definitely more by the presence of Co^{2+}.

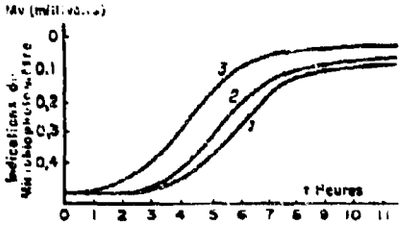

Fig. 4. Growth curves of Cl. sporogenes in a complex medium. Curve 1 = control culture; Curve 2 = culture in the presence of Ni (0.8 X 10^{-3} mol); Curve 3 = culture in the presence of Co (0.8 X 10^{-3} mol).

/Legend:/ Vertical scale as in Figure 2, horizontal coordinate as in Figure 1.

DISCUSSION

Aerobes and anaerobes differ notably by the mechanism of their oxidations. While aerobes are able to use the molecular oxygen of the air; anaerobes accomplish their oxidations with the aid of indirect chemical processes that the presence of O_2 inhibits.

Molecular oxygen must be activated in order to be utilizable in cultures of aerobic germs. Oxidation reactions in vivo are often enzymatic. But biological oxidation reactions, both in vivo and in vitro, can be activated or inhibited by such strongly electrophile agents as metallic cations.

The most usual reactions accomplished by aerobic bacteria with the aid of O_2 are:

$$R\begin{matrix}OH\\OH\end{matrix} + O_2 \xrightarrow{M^{2+}} R\begin{matrix}=O\\=O\end{matrix} + H_2O_2$$

Transformation, for example, of phenols into equinones (Cu^{2+}, Mn^{2+})

$$RSH + RSH + O_2 \xrightarrow{M^{2+}} RS\text{-}SR + H_2O_2$$

Formation of S-S bonds in the synthesis of proteins (Fe^{2+}, Mn^{2+})

In both of these oxidation reactions, certain metallic cations are active, while others are inhibitory. That is demonstrated in the first case by an increase in syntheses, whence an acceleration in growth and, in the second case, by a decrease or a suppression of the exponential phase of the microbian multiplication.

These active metallic cations belong to the transition metals. They have non-saturated penultimate electronic orbits, which allows them to receive pairs of bonding electrons from coordinator atoms each one of which has a pair of free electrons for bonds by coordination. Then a complex of coordination is formed, which may be a chelated complex, like the one obtained with two glutathione molecules.

Moreover, in order for the central cation to be the activator of the oxidation reaction of the coordinated molecule, or molecules, it must be able to receive or give up one electron. This electron then is used to make the oxygen act on the coordinated molecule, or molecules, through the intermediary of the central metallic cation. Thus a bond is established by resonance between the oxygen molecule and the

coordinated substrate molecules. A theoretical study from the point of view of electronic chemistry has been made (Goudot and Faguet [3]). From the point of view of general chemistry, that is demonstrated by the transformation of neutral molecules into ions with contrary signs which are then able to react within the coordination complex that is then the "transition complex" or "activated complex" (Fig. 7).

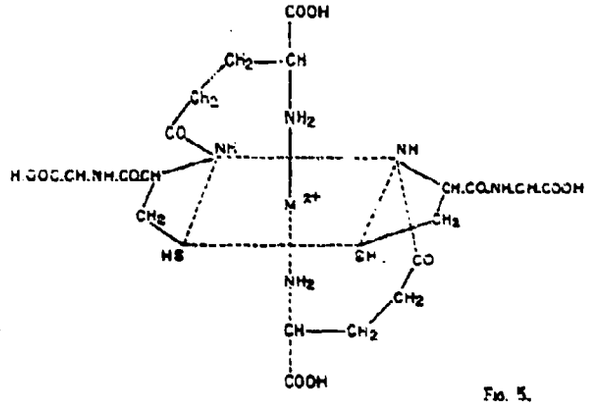

Fig. 5.

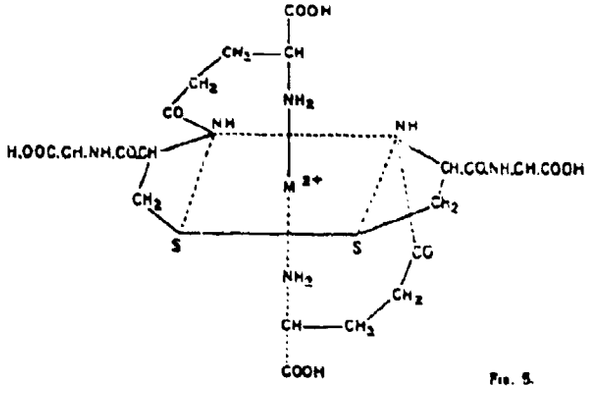

Fig. 6.

Figures 5 and 6: Charge transfer complex: metal substrates

Fig. 5. represents the initial complex: divalent cation and two substrate molecules: M^{2+} - 2GSH + O_2.

Fig. 6. (Caption on following page)

Fig. 6 represents the complex after the reaction: divalent cation and formation of the S-S bond: M^{2+} - GS - SG + H_2O_2. An intermediate activated state is found between these two states: M^{3+} - 2GSH - O_2^-.

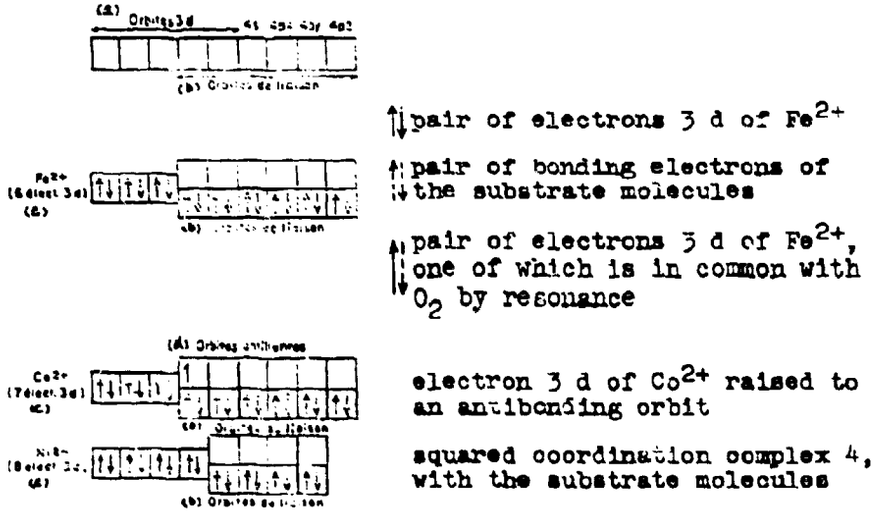

Fig. 7. Formation of coordination complex with the transition elements

[Legend:] a) 3 d orbits; b) bonding orbits; c) electr.; d) antibonding orbits.

In the reactions given above, there is, with the aid of activator cations, displacement of one electron of the metallic cation on O_2, while within the complex formed with the molecules to be oxidized, there is a displacement of the mobile electrons which produces a positive charge on the S and H atoms, assisting in dissociating the SH bond. Thus Fe^{2+} and Mn^{2+}, by displacing an electron on O_2, produce the oxidation reaction of the SH groups. The formation of S-S gives back the electron displaced on the transitory trivalent metallic cation. Ni^{2+} is an inhibitor, for it does not fix O_2. On the other hand, Co^{2+} produces ionization of O_2. It does not permit an interaction between the substrate and the activated oxygen O^2, but if this activated oxygen is released, it is able to act on the free molecules of thioprotein. This explains why our cultures of E. coli display a

weak growth in the presence of Co^{2+}, whereas in the presence of Ni^{2+} they are completely arrested.

In the case of anaerobic bacteria, the same cations play an inverse role in the nutrient medium, in the presence of O_2, regardless of whether the molecular oxygen is in a free state of whether it is produced at the time of intermediary reactions. This is important, because CO_2 is often a reagent of anaerobic reactions. Now, O_2 displaces CO_2 to fix itself on the metallic cation of the complex. Therefore, in metabolic reactions, the active cations of aerobic syntheses become inhibitors. Ni^{2+}, which does not fix O_2, but prevents it from accomplishing oxidation reactions, must allow the syntheses that have to be made in the absence of O_2. Therefore, it must increase the growth of cultures of anaerobic bacteria.

CONCLUSIONS

Our experiments show that there is agreement, in the broad sense, between theory and results. Nevertheless, it is necessary to take into account the fact that a great number of reactions are produced simultaneously in the mechanism of microbian multiplication. A cation that activates a synthesis reaction may, at the same time, activate dissociation reactions. The observed growth, then, is the result of two opposite processes. The more rapid one will predominate. That is how Mn^{2+} acts on an activator in the S-S synthesis reactions. But, on the other hand, it is active in the dissociation of the peptidic bond. By increasing the amount of Mn^{2+} in the nutrient medium, at the same time the synthesis of the S-S bonds and the rupture of peptidic bonds is accelerated in the synthesis of the thioproteins.

SUMMARY

We have sought to predict, by studying different reactions that are produced during the growth of cultures of aerobic germs (E. coli) and anaerobic germs (Cl. sporogenes), the way in which cations of different metals (Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}) act on the multiplication of these germs.

The experimental results turn out, on the whole, to be in agreement with the predictions. In a synthetic medium with glucose, Fe^{2+} and Mn^{2+} favor the growth of E. Coli, Co^{2+} decreases it by 50% and Ni^{2+} inhibits it completely

(~~concentrations~~ of 0.5 X 10^{-5} ~~mol~~), while Ni^{2+} and Co^{2+} stimulate the growth of an anaerobic germ (Cl. sporogenes), cultivated in a complex medium with glucose (~~concentration of 0.8 X 10^{-3} mol~~).

BIBLIOGRAPHY

/1/ FAGUET, M., Ann. Inst. Pasteur, 97, 1959, pp 177-187.

/2/ FAGUET, M., Ann. Inst. Pasteur, 100, 1961, pp 594-599.

/3/ GOUDOT, A. and FAGUET, M., C. R. Acad. Sci., 252, 1961, pp 2557-2559.

/4/ MONDO, J., Croissance des cultures bacteriennes (Growth of Bacterial Cultures), Paris; Hermann et Cie., 1942, 17, No 911.

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