CLXXX. PRELIMINARY NOTE ON THE ACTION OF GASES UNDER PRESSURE ON THE GROWTH OF MICRO-ORGANISMS.

I. ACTION OF OXYGEN UNDER PRESSURE AT VARIOUS TEMPERATURES.

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THE first observations on the action of oxygen at high pressure on the respiratory functions of animals and plants were made by the French physiologist Bert [1872; 1873, 1, 2]. He found that the consumption of oxygen by animals placed in oxygen under 3·5 atmospheres' pressure, or in air under correspondingly higher pressures, fell far short of the normal and that such animals succumbed rapidly with symptoms resembling those of strychnine poisoning.

The subjects of putrefaction and of fermentation were foremost in the minds of many biologists at that time, and Bert extended his researches from the behaviour of higher animals and plants to that of the action of high pressure oxygen on putrefaction.

In a final paper Bert [1875] reported that he had succeeded in keeping a piece of raw meat free from putrefaction for a prolonged period by storing it in a container under an oxygen pressure of 15 atmospheres. Raw eggs were similarly preserved. In the case of the meat the oxygen affected the colour, turning the normal red into a yellowish amber, while the taste of the meat became somewhat flat.

Bert [1875] also attempted to ascertain what effect, if any, oxygen under pressure would have on various enzymes. He concluded from his observations that vegetable diastase, pepsin and the enzymes of saliva and of the pancreatic juice remain unaffected under these conditions. Further, he pointed out that the protective action of oxygen and of air under high pressures was a function of the abnormally increased concentrations of oxygen which entered the living tissues under these conditions and not of the pressures as such. In other words he believed the action to be one typical for oxygen and not necessarily inherent in other gases.

Subsequently Nourry and Michel [1892] reported that milk, kept in the cold and under a high pressure of carbon dioxide, would remain sweet for as long as 8 days owing to a retarding action of the carbon dioxide on the growth of the microflora of the milk.

An inhibitory action of carbon dioxide on micro-organisms was observed also by Hoffmann [1906] who claimed that *Bact. coli commune*, *Bact. typhosum* and *Vibrio cholerae* could be destroyed when subjected to carbon dioxide pressures of from 20 to 50 atmospheres. Hoffmann's observations were followed up by Berghaus [1907] who concluded that carbon dioxide as such, even in the

absence of high pressures, has an inhibitory action on many micro-organisms, an action which increases with the increased quantities of the gas brought into solution on the application of high pressures.

Curiously enough Berghaus reported that the action of carbon dioxide under pressure in his experiments was very much more marked than that of oxygen similarly applied. Organisms like *Bact. coli commune* and *Staphylococcus pyogenes aureus* showed retarded growth when incubated at 37° under an oxygen pressure of up to 75 atmospheres while 15 atmospheres and 9 atmospheres respectively of carbon dioxide applied for 24 hours at 37° were found sufficient to destroy these micro-organisms.

Incomplete and inconclusive as were the above observations they formed the basis for various attempts to apply gases under high pressures for purposes of sterilisation.

The first attempt after that of Bert to apply high pressure gases for purposes of sterilisation was made by d'Arsonval [1891] who claimed to be able to sterilise liquids by passing them through porcelain candles, using high pressure carbon dioxide gas to facilitate the filtration and to weaken the micro-organisms present in the liquids. The weakening of the micro-organisms, he found, was particularly marked when the filtering liquid was kept at increased temperatures (40°).

Schaffer and von Freudenreich [1892] were unable to confirm d'Arsonval's conclusions and report that they failed to sterilise milk by exposure to high pressures of oxygen and carbon dioxide, even when raising the temperature of the milk considerably above 40°. They asserted that even *Bact. typhosum* and vegetative cells of *B. anthracis* remained viable when exposed in milk under such conditions. It should be pointed out that Schaffer and von Freudenreich carried out their experiments under insufficiently controlled conditions, and that their conclusions therefore appear unsupported experimentally.

De Lavallée [1898] obtained more satisfactory results and claimed that he had succeeded in preserving milk for a considerable time and in freeing it from pathogenic micro-organisms by subjecting it first to an exposure for 4–5 hours to a carbon dioxide pressure of from 5 to 6 atmospheres and then to an oxygen pressure of 5 atmospheres for 5 hours. In his experiments the milk was apparently kept cool, since it was taken direct from the cow into cooled containers and then treated. De Lavallée's results were repeated on various occasions but could not be corroborated and Seiffert [1904], Gerasbeck [1905] and Riegel [1905] reported unfavourably on the use of high pressure gases for the sterilisation of milk. Gradually interest in the subject slackened, and little was heard of the bactericidal properties of high pressure gases until Cleveland's [1925] publications on the intestinal fauna of termites, though papers appeared from time to time which testified to the growth-inhibiting action of oxygen on certain types of micro-organisms. Of special interest in this respect are Moore and Williams's [1911] observations that *Mycobacterium tuberculosis* is killed when kept in an atmosphere containing 80 % of oxygen, in conditions in which *Staphylococcus pyogenes aureus* is merely retarded in its development.

Cleveland [1925] succeeded in eliminating the intestinal protozoa of termites by exposing the insects to oxygen pressures of 3·5 atmospheres for periods insufficient to destroy the host itself. He obtained similar results when using air under correspondingly higher pressures, but failed to destroy the protozoa completely when using an air pressure of only 3·5 atmospheres, thus showing that the destructive action was associated with the oxygen and not with the pressure as such. In this respect he confirmed earlier observations, particularly

those of Rogers [1894] who found that pressures of 1000 atmospheres and more may be applied to bacterial cultures without detrimental effect.

In a brief note published subsequently, Cleveland [1926] drew attention to the possibility of utilising the destructive properties of oxygen under pressure for various microbiological purposes such as the sterilisation of culture media. In other words Cleveland reopened the problem of the bactericidal action of high pressure gases in the form in which it had been left by Bert and without, apparently, being aware of the fact that a considerable amount of work had already been done which tended to prove that the destructive properties of high pressure gases are insufficient to justify their practical application. On the other hand it must be admitted that the adverse criticism published prior to Cleveland's investigations was of a very desultory nature.

It is particularly noteworthy to observe when glancing through the earlier observations on the bactericidal action of high pressure gases, that no systematic attempt was ever made to ascertain the relationship of the action of the gas to the various factors which affect the development of all living cells, notably time and temperature, and it was felt that if, as suggested by Cleveland, the question of the use of high pressure gases should be reopened, one of the first investigations to be undertaken would have to be an analysis of the extent to which the duration of the exposure to the gas and the temperature at which the exposure is carried out would affect the bactericidal properties of high pressure gases.

EXPERIMENTAL.

In the curves shown below certain results have been tabulated, taken from a number of preliminary experiments which were carried out to ascertain the effect of temperature on the action of high pressure oxygen. In these experiments four different types of micro-organisms were used, ranging from a typical aerobic *Bacillus* to a semi-anaerobic *Streptococcus*. For comparison, curves have been plotted also of the growth of the same organisms under identical conditions but left at the test temperatures under ordinary atmospheric conditions.

The curves record the percentage increase and decrease respectively in the numbers of cells present in the cultures in question after 5 hours' incubation at the temperatures recorded on the abscissae, either at ordinary atmospheric pressure or under an oxygen pressure of 10 atmospheres.

It will be observed that in all four cases the high pressure oxygen has had a retarding influence on the growth of the test organisms. But the most noteworthy feature of the curves is that, while at temperatures well below the optimum temperature for growth, the action of the oxygen has been unable to prevent an actual, and sometimes an appreciable, increase in the initial number of cells, a marked decrease has set in where the optimum temperature for growth at atmospheric pressure has been reached or slightly exceeded.

Thus in the case of the vegetative cells of *Bac. mesentericus* (Fig. 1) the raising of the temperature from 24° to 30° has changed a slight but distinct increase in the initial number of cells to a complete destruction of all but the spores present. Of the spores only a small percentage was found to be destroyed by the high pressure oxygen.

In the case of *Bact. coli commune* (Fig. 2) kept under 10 atmospheres' oxygen pressure, it took an increase in temperature of 13°, from 34° to 47°, to change a marked increase in numbers to complete destruction. But this complete destruction was nevertheless achieved at a temperature when the corresponding control culture, kept at atmospheric pressure, still showed an increase in the initial number of cells of nearly 100 %.

Similar, though perhaps somewhat less marked, results were obtained with the two other types of organisms used, *Streptococcus lactis acidi* (Fig. 3), and *Staphylococcus pyogenes aureus* (Fig. 4). In all the four cases it was possible to secure complete destruction within 5 hours of the vegetative cells originally

Fig. 1.

Fig. 1. *Bac. mesentericus* (vegetative cells) suspended in ordinary broth (p_H 7.1).

Curve A. Percentage increase (decrease) of the original number of cells after 5 hours' exposure to air (atmospheric pressure) at temperatures shown.

Curve B. Percentage increase (decrease) of the original number of cells after 5 hours' exposure to oxygen (10 atmospheres' pressure) at temperatures shown.

Fig. 2.

Fig. 2. *Bact. coli commune*; suspended in 1 % glucose broth (p_H 7.1).

Curve A. Percentage increase (decrease) of the original number of cells after 5 hours' exposure to air (atmospheric pressure) at temperatures shown.

Curve B. Percentage increase (decrease) of the original number of cells after 5 hours' exposure to oxygen (10 atmospheres' pressure) at temperatures shown.

introduced into the culture at a temperature of 47°, a temperature which was insufficient to eliminate all the initial cells in the controls kept at atmospheric pressures and which should leave coagulable protein and presumably other thermolabile substances unimpaired.

The curves given illustrate why such divergent results as those referred to above should have been obtained by earlier investigators, who, in the majority of cases, were satisfied with testing the action of high pressure oxygen at lower

Fig. 3.

Fig. 3. *Streptococcus lactis acidii*; suspended in 1 % glucose broth (p_H 7.1).

Curve A. Percentage increase (decrease) of the original number of cells after 5 hours' exposure to air (atmospheric pressure) at temperatures shown.

Curve B. Percentage increase (decrease) of the original number of cells after 5 hours' exposure to oxygen (10 atmospheres' pressure) at temperatures shown.

Fig. 4.

Fig. 4. *Staphylococcus pyogenes aureus*; suspended in broth (p_H 7.1).

Curve A. Percentage increase (decrease) of the original number of cells after 5 hours' exposure to air (atmospheric pressure) at temperatures shown.

Curve B. Percentage increase (decrease) of the original number of cells after 5 hours' exposure to oxygen (10 atmospheres' pressure) at temperatures shown.

temperatures, *i.e.* at room temperature or below. Why Schaffer and von Freudenreich [1892], who used a temperature as high as 68°, should have failed in their attempts to destroy the non-spore-forming bacteria of samples of milk which they subjected to oxygen pressures is not explained by the curves. But other

experiments carried out by the writer on the influence of the time factor on the bactericidal action of high pressure oxygen appear to indicate that Schaffer and von Freudenreich's negative results were due to the fact that they failed to maintain the increased temperatures for a sufficient time to secure complete destruction. However, before expressing a definite opinion on this point, it will be necessary to carry out a closer study of the bearing of the time factor on the bactericidal action of high pressure oxygen, a study which has not yet been completed.

The present preliminary note was compiled for the purpose of showing that it is possible to confirm experimentally the early observations of Bert and others who attributed bactericidal properties to oxygen at high pressure, and of drawing attention to the need for a careful and complete investigation of the whole of this question with a view, as suggested by Cleveland [1926], to elaborating methods for the destruction of micro-organisms in all such cases where the application of heat or antiseptics would affect the composition of substances such as proteins, enzymes, vitamins and hormones.

SUMMARY.

It has been shown that the following test organisms, *Bac. mesentericus*, *Bact. coli commune*, *Streptococcus lactis acidi* and *Staphylococcus pyogenes aureus*, are very greatly retarded in their growth when this is made to take place in an oxygen atmosphere of 10 atmospheres' pressure at or below the optimum temperature for growth.

On increasing the temperature for growth slightly, by a few degrees, and in some cases as much as 13°, above the optimum temperature for growth the effect of oxygen under pressure on the test organisms was found to be lethal, the gas completely destroying all vegetative cells present in 5 hours or less.

The spores of the strain of *Bac. mesentericus* used were not killed under these conditions.

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