CXLIII. EXPERIMENTS ON "STRICT" ANAEROBES.

I. THE RELATIONSHIP OF *B. SPOROGENES* TO OXYGEN.

By JUDA HIRSCH QUASTEL (1851 *Senior Exhibitioner*)
AND MARJORY STEPHENSON.

From the Biochemical Laboratory, Cambridge.

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In the course of an investigation into the nutritional requirements governing the growth of the "strict" anaerobes, as typified by *B. sporogenes*, two factors have come to light. The first of these conditions the relation of the cell to oxygen; this alone will be dealt with in the following communication. The second is concerned with nutrition and continued reproduction and will be dealt with in a later communication.

Since the discovery by Pasteur [1861] of the existence of organisms "vivant sans gas oxygène libre" the relationship of the true anaerobes to oxygen has been obscure. Pasteur himself considered that air killed them.

The toxic effect of oxygen has been generally accepted since Pasteur's day and numerous attempts have been made to explain this phenomenon. Recently McLeod and Gordon [1923] have observed that anaerobes contain little or no catalase, and they have attributed the toxic effect of oxygen to the production of hydrogen peroxide with which the cell, in the absence of catalase, has been unable to cope.

Callow [1923] has expressed the same conviction and supports it by showing that the anaerobes possess little or no catalase so that "in the absence of catalase the hydrogen peroxide would accumulate and kill the cell." She has stated, however [1924], that factors other than lack of catalase may play a part with the anaerobes. Sherman [1926] has noted that certain obligate anaerobes possess catalase.

Avery and Morgan [1924] find that the presence of sterile unheated plant tissues in a plain broth induces the aerobic growth of certain anaerobes and attribute the effect to decomposition of peroxide by the catalase and peroxidases of the plant. They consider that the failure of Callow and of McLeod and Gordon to obtain aerobic growth of anaerobes in media containing catalase is due to the possibility that "the complete oxidation-reduction system in the fresh plant tissue is able to decompose toxic peroxides of an order unaffected by catalase alone." McLeod and Gordon [1925, 2] assert that because bacteria possess reducing powers they are capable of giving rise to hydrogen peroxide and give indirect evidence [1925, 1] for the formation of this peroxide during the aerobic growth of some anaerobes.

This interpretation of the effect of oxygen is not accepted by Novy [1925] who has observed that the aerobic growth of anaerobes can be effected in an *h* tube by a distant culture of *B. subtilis* or by distant potato, both of which reduce the tension of oxygen by their aerobic respiration until an oxygen-free environment is established. The anaerobes then multiply. Thus, when oxygen is present in a medium inoculated with an anaerobe, proliferation does not occur; if oxygen be then removed mechanically, as by reduction of tension, multiplication of the organisms begins.

Hence the failure of anaerobes to grow aerobically cannot be due to a lethal action of oxygen or to the suicidal production of hydrogen peroxide; they appear rather to be in a state of suspended animation and apparently, from Novy's experiments, await only removal of the oxygen to multiply once more. The initial problem, therefore, of determining why oxygen exerts an inhibitory influence on the anaerobes still remains unsolved.

Novy suggests that the strict anaerobe depends for its proliferation on an enzyme—an anaerase—which can only work in the absence of oxygen. The suggestion is not very satisfactory for it postulates the existence of an enzyme which must be of such a nature that it becomes oxidised, or functionless, in the presence of oxygen and reduced, or capable of re-functioning, when the oxygen is removed—even when the process of removal is mechanical as by reduction of tension. We have no evidence for the existence of such an enzyme and later we will give results which make the suggestion untenable.

Preliminary observations.

As is well known, the growth of *B. sporogenes* on the usual nutrient media is accompanied by an offensive smell, partially, if not wholly, due to the formation of a number of sulphydryl compounds. We have performed numerous experiments in connection with the nutrition of the organism and we have found generally that the growth of the organism is accompanied by substances containing the —SH group[1], this either being liberated from, or present initially in, the medium.

Our attention was focussed in the first place on the significance of the —SH group in connection with the growth of *B. sporogenes*, by the following observation. A culture of the organism in tryptic broth had been allowed to stand exposed to air and room temperature for several days, after which time the culture had almost entirely lost its smell and ability to give a nitroprusside reaction. Inoculation of two drops of the culture into a freshly autoclaved tryptic broth medium, followed by incubation under strictly anaerobic conditions at 37°, failed to produce a growth within 20 hours, whilst the same inoculation into a freshly autoclaved tryptic broth containing 0·1 % neutralised cysteine hydrochloride resulted in a profuse growth of the organism. Further investigation showed that this property of inducing rapid growth of *B. sporogenes* was shared not only by cysteine but by thioglycollic acid and glutathione.

[1] As detected by the nitroprusside reaction.

Very small concentrations of these substances were equally effective and it was clear that the phenomenon was connected with the —SH group and not with any nutritional effect of the molecules as a whole. *B. sporogenes*, it may be added, fails to develop in a medium whose carbon or nitrogen is solely supplied by cysteine.

In our experimental work we have almost entirely confined ourselves to the use of cysteine to provide the —SH group. It is easily prepared in a state of purity and, in the form of its hydrochloride, is stable. It is easier to handle than thioglycollic acid, whilst the preparation of glutathione is too laborious and expensive to make this substance convenient for bacteriological purposes.

Media employed.

Tryptic broth was made according to Cole and Onslow's method [1916] and was used at a concentration containing 3 mg. N per cc. For work with *B. sporogenes* it was made up (with NaOH) to an initial p_H of 7·8.

The crude cysteine hydrochloride (prepared from hair) was recrystallised from alcohol and chloroform. It was added to tryptic broth so as to bring the concentration to 0·1 %, made up to p_H 7·8 with sodium hydroxide and immediately autoclaved. It will be convenient to refer to a tryptic broth medium as TB and to a tryptic broth medium containing 0·1 % cysteine hydrochloride as TB—CSH. The media were inoculated usually not longer than 3 hours after their preparation and sterilisation. Cysteine, it should be observed, oxidises fairly rapidly at p_H 7·8 and it is desirable to inoculate the media as quickly as possible after the preparation and sterilisation. Control experiments to exclude the possibility of contamination having occurred were always performed. 5 cc. of medium per tube were usually employed.

Exp. 1. Two or three drops of an 18-hour culture of *B. sporogenes* in tryptic broth were inoculated into TB and TB—CSH and incubated aerobically at 37°. After 18 hours there was no growth in TB but a heavy one in TB—CSH. If a large inoculation of a fresh culture of *B. sporogenes* be sown into TB, growth may occur aerobically, but this, we find, is generally associated with the fact that with a large inoculation a considerable quantity of —SH[1] is transferred along with the culture to the new medium. If little or no —SH is carried over, aerobic growth of *B. sporogenes* does not occur; good aerobic growth, on the other hand, takes place in TB—CSH. This phenomenon will be referred to in more detail later. Trenkmann has shown [1898] that the presence of alkali sulphide will induce aerobic growth of anaerobes.

Cysteine, thus, offers a contrast to catalase, which will not induce aerobic growth of the anaerobes. If the suggestion be right that the failure of catalase to induce aerobic growth is due to its inaccessibility to the peroxide formed within the cell, then it can be maintained that the success of cysteine is due either (1) to its preventing oxygen reaching the cell or (2) to its ability to reduce intracellular peroxide.

[1] It is very convenient to speak of —SH as though it were a separate entity.

Exp. 2. A culture of *B. sporogenes* in TB was exposed to pure oxygen for 6 hours at 37° and two drops were inoculated into TB and TB—CSH respectively. After 18 hours' incubation under strictly anaerobic conditions, the TB had failed to produce a growth whereas a prolific one was present in TB—CSH. These were then incubated anaerobically for a further 24 hours, at the end of which time a copious growth was present in TB.

On the peroxide theory it can be argued that exposure to oxygen has produced peroxide either in the organism or in the culture medium; hence no growth occurs in TB but a good one is present in TB—CSH where the cysteine removes the peroxide. But since growth occurs in TB—CSH it is clear that the effect of the oxygen (or peroxide produced by oxygen) is not lethal. Again, in TB alone growth developed after 42 hours' anaerobic incubation. This must mean on the peroxide theory that the peroxide produced by exposure to oxygen is simply removed by the broth itself, the effect of the presence of cysteine being to hasten this removal. But it is clear that the peroxide produced by exposure cannot have been fatal, otherwise no growth would have developed whether —SH were present or not and however long the incubation.

It may be urged that the effect of the oxygen has been to kill a very large proportion of the organisms, the long lag in growth in TB being due to the slow development of the survivors. This does not account for the fact, however, that the latent period almost entirely disappears in presence of CSH.

Exp. 3. 100 cc. of TB—CSH were placed in ten test-tubes, sown with a fresh culture of *B. sporogenes* and incubated aerobically for 24 hours. A copious growth in each tube resulted. The cultures were poured into sterile centrifuge tubes which were then covered with guttapercha caps, centrifuged and the crop of *B. sporogenes* washed once with sterile saline. It was then emulsified in 50 cc. saline. 20 cc. of this emulsion were placed in a small sterile wash-bottle and filtered air was bubbled through at room temperature for 4 hours. After this period two drops of the emulsion were inoculated into TB, TB—CSH and TB containing 1 % glucose (the glucose having been autoclaved separately). After 20 hours' anaerobic incubation, no growth was apparent in TB or TB-glucose but a profuse one in TB—CSH.

Exp. 4. An emulsion of *B. sporogenes* was made up as in the previous experiment, 10 cc. placed in a sterile wash-bottle and oxygen passed through the emulsion at room temperature for 6 hours. Three drops were inoculated into TB and TB—CSH. After 16 hours' anaerobic incubation there was little, if any, growth in TB but a prolific one in TB—CSH. Another 10 cc. of *B. sporogenes* were treated in a similar way with nitrogen and another with hydrogen. Both these emulsions after this treatment gave a good growth in TB, after 16 hours, as well as in TB—CSH. To another 10 cc. of the initial emulsion of *B. sporogenes*, neutral sterile cysteine hydrochloride was added to a concentration of 0·1 % and oxygen bubbled through for 6 hours. Anaerobic subculture showed, in 16 hours, a questionable growth in TB but a prolific one in TB—CSH.

Exp. 5. A culture of *B. sporogenes* in TB—CSH was bubbled with oxygen for 7 hours at room temperature. Subculture showed, after 18 hours' anaerobic incubation, a slight growth in TB but an excellent one in TB—CSH.

We may conclude quite definitely from the foregoing results that (1) oxygen is not fatal to *B. sporogenes*, (2) if oxygen gives rise to peroxide, this is only produced at a concentration small enough to affect proliferation but not to kill the organism. So far our results are in agreement with Novy's observations.

Exp. 6. 50 cc. of an emulsion of *B. sporogenes* was prepared as before and through this oxygen was passed for 24 hours at 37°. This emulsion was then divided into four parts, *A*, *B*, *C* and *D*, which were treated as follows:

(*A*) a rapid stream of hydrogen was run through to remove free oxygen;

(*B*) a stream of hydrogen sulphide was run through for 4 hours to remove oxygen and possibly peroxides. Hydrogen was finally run through to remove hydrogen sulphide;

(*C*) sterile neutral cysteine hydrochloride was added to a concentration of 0·1 % and the emulsion kept anaerobically at 37° for 4 hours. It was then centrifuged to remove —SH and the deposit of organism made up to the initial volume with sterile saline;

(*D*) the same procedure as in (*C*) was adopted but thioglycollic acid was substituted for cysteine.

The four emulsions were then inoculated into TB and TB—CSH and incubated anaerobically. The growths were examined after 18 hours and 66 hours. They are noted in Table I.

Table I.

Medium	TB	TB—CSH	TB	TB—CSH	TB	TB—CSH	TB	TB—CSH
Inoculant	*A*	*A*	*B*	*B*	*C*	*C*	*D*	*D*
Growth after 18 hours	0	+ + +	0	+ + +	0	+ + +	0	+
Growth after 66 hours	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +

These results show that the effect of prolonged exposure of *B. sporogenes* to oxygen is to cause a retardation of growth in TB (*i.e.* no growth in 18 hours but positive growth in 66 hours).

Removal of free oxygen from the organism mechanically as by means of hydrogen or chemically as by an —SH group does not immediately restore the cell to its initial condition. Oxygen must therefore affect the organisms in some way which is not *immediately* reversed by the removal of the oxygen. Novy's suggestion, which implies that the removal of free oxygen will result in immediate restoration of the anaerase and hence of the power of the organism to proliferate is, therefore, not supported.

The retardation of growth brought about by oxygen is not due to the presence of peroxides in the cell for the preliminary treatment of the oxygenated organism with —SH, which would have removed such substances, had no pronounced effect in restoring the immediate growth of the organism.

Now, since the lack of growth of the oxygenated organism in TB during the first 18 hours is not due to a lethal action of the oxygen, nor to the presence

of oxygen or peroxides in the inoculant, it follows, since good growth occurs in the same medium after 66 hours that, during the long latent period, either the inoculant or the medium has changed towards a condition favourable to anaerobic growth, and that the presence of —SH initially (as in TB—CSH) either greatly accelerates this change or, indeed, is itself responsible for the change. It is unlikely that tryptic broth alone will alter its composition during anaerobic incubation at 37°; the change, therefore, must be due to the inoculant. Since we know (1) that the presence of very little —SH will almost entirely remove the long latent period, and (2) that the growth of *B. sporogenes* on TB generates —SH compounds so that it is evident the organism possesses a proteolytic mechanism capable of producing —SH from TB, it appears reasonable to assume that the long latent period exhibited by the oxygenated organism is associated with the slow production of an —SH body, this being produced by the proteolytic activity of the inoculated cells and proliferation occurring immediately this —SH has reached a definite concentration. If this assumption is true we should be able to observe a powerful effect of —SH in a medium which initially contains no organic sulphur. For experiment we have used a digest of gelatin as one which initially is practically devoid of organic sulphur, and we have compared it with a digest of caseinogen (prepared similarly).

Exp. 7. Gelatin and caseinogen were completely hydrolysed with 20 % sulphuric acid for 30 hours, the sulphuric acid removed by baryta and the two digests neutralised and made up into stock solutions. They were used at concentrations containing 3 mg. N per cc. and at an initial p_H of 7·8. Phosphates and a little sulphate were added to them. Table II shows the effect of 0·1 % cysteine hydrochloride on the anaerobic growth of *B. sporogenes* in each medium. Two drops of a fresh culture of the organism were inoculated.

Table II.

Medium	Gelatin digest	Gelatin digest + CSH	Caseinogen digest	Caseinogen digest + CSH
Anaerobic growth after 16 hours	0	+ + +	+ + +	+ + +

Thus in a medium such as an acid digest of gelatin containing practically no organic sulphur, from which the cell can manufacture —SH, no anaerobic growth occurs unless —SH is artificially added, whereas in acid digest of caseinogen which—like tryptic broth—contains no initial —SH but a source of organic sulphur from which the organism can make it, good anaerobic growth occurs. An addition of CSH, however, supplies the necessary factor to the acid digest of gelatin.

Table III shows the effect of inoculating *B. tetanomorphus* into gelatin digest and tryptic broth in the presence and absence of either cysteine or of 1 % glucose. (The glucose was autoclaved separately.)

Table III.

Medium	Gelatin digest	Gelatin digest + CSH	Gelatin digest + glucose	TB	TB + CSH	TB + glucose
Anaerobic growth after 3 days ...	0	+ +	0	+ + +	+ + +	+ + +

The influence of —SH appears to be much greater than that of glucose. We have observed, however, that the addition of glucose to a gelatin digest at p_H 7·8 with subsequent autoclaving does produce a medium fairly favourable to the anaerobic growth of *B. sporogenes*. In performing these experiments great care must be taken not to carry over with the inoculant an appreciable quantity of —SH.

A hypothesis which will satisfactorily interpret the action of oxygen must account for the following facts:

(1) that a fresh culture of *B. sporogenes* inoculated into TB exhibits a relatively small latent period in its anaerobic growth;

(2) that a culture of the organism, oxygenated for some time, exhibits when cultivated under strictly anaerobic conditions, a long latent period in TB but not in TB—CSH;

(3) that this long latent period is not due to the presence of oxygen or peroxides in the inoculant.

We have suggested that this latent period may simply be the time of incubation necessary for the inoculated non-proliferating cells of *B. sporogenes* to produce, by proteolytic activity, a minimal quantity of —SH. As soon as this amount is formed proliferation commences. The results with gelatin support this suggestion.

A ready explanation for the behaviour of oxygen is now forthcoming; for if the —SH formed by the organism from TB is removed by oxygen at a rate at least equal to the velocity of its formation then the minimal concentration of —SH is never produced and proliferation cannot occur. If —SH be initially present (as in TB—CSH) then not only will the —SH protect the cell from oxygen, for a time dependent on its concentration, but it will secure conditions for immediate proliferation of the inoculated organism. Such proliferation will result, by proteolytic activity, in the production of more —SH. Thus, even if the initial quantity of —SH in the medium were such as to require relatively little time for its oxidation, yet in this time proliferation may have commenced with the consequent production and maintenance of a steady supply of —SH from the medium. The problem, indeed, resolves itself into one of relative velocities; if the velocity of oxidation of the —SH be greater than the rate of its formation, then proliferation cannot occur; if proliferation has already commenced by reason of the initial presence of a quantity of —SH then proliferation will continue as long as the minimal concentration of —SH is maintained.

We do not, at present, however, consider that only the —SH group has this effect in securing the condition necessary for the proliferation of *B. sporo-*

genes. Our experience points to the fact that glucose also may have an effect similar to —SH, but the effect seems, in the case of *B. sporogenes*, initially, to be much less powerful.

A most important fact concerning the —SH group (as illustrated by cysteine and glutathione) is its ability to give a reduction potential which is not influenced by the presence of the oxidised (—S—S—) form. This, it appears to us, is the significant fact underlying the phenomena we have described. Even very low concentrations of cysteine, as an examination of the relationship between reduction potential and concentration of this substance [Dixon and Quastel, 1923] will indicate, give high reduction potentials; solutions so dilute that the nitroprusside reaction is indefinite give relatively high potentials. In view of this fact we may restate the hypothesis put forward earlier.

Let us assume that before proliferation of *B. sporogenes*—or of any cell which leads a "strictly" anaerobic career—can occur, the cell must have attained a certain limiting reduction potential. At any potential more oxidising than this the cell fails to proliferate. At or below this potential proliferation is possible. The potential may be secured by the presence of a small quantity of an —SH compound or it may obtain in the presence of glucose or a variety of substances characterised by their reducing behaviour in presence of the cell.

The effect of oxygen in preventing the proliferation of strict anaerobes would then be due to its removal of substances responsible for the maintenance of the critical reduction potential with the consequent raising of the potential above the limiting point.

A fresh culture of *B. sporogenes* is at or below its limiting reduction potential and hence its inoculation into a medium[1] which will not markedly affect this potential will be followed by a relatively rapid proliferation. Its inoculation, however, into one which increases the potential above the limiting point will result in failure to proliferate, until the organism has, itself, by its proteolytic (or other) activity, induced the formation of substances creating the requisite limiting potential. Similarly, a culture of *B. sporogenes* which has been oxygenated, or treated in some such way, will possess a potential above its critical point and its inoculation into an inert (*i.e.* from the point of view of affecting the potential) nutritional medium will result in the manifestation of a long latent period in its anaerobic growth. On subculture, however, into a medium already possessing a high reduction potential (*e.g.* TB—CSH) this latent period will be diminished or removed. The latent period, it is clear, is a function of (1) the nature of the medium or environment, (2) the power of the cell to produce substances creating a reduction potential, (3) the value of the cell's characteristic limiting reduction potential.

Potentiometric evidence, such as that obtained by Cannan, Cohen and

[1] It is assumed of course that the medium possesses the nutritional requirements of the organism.

Clark [1926] with certain organisms, would, probably, be of considerable value in this connection.

We may now consider the effects of exposing an anaerobe such as *B. sporogenes* to hydrogen peroxide. There is little doubt that in a growing culture of *B. sporogenes* containing —SH compounds and exposed to the air, there is formation of hydrogen peroxide. McLeod and Gordon's observations [1925, 1, 2], taken together with the recent work on oxidations in presence of cysteine or glutathione, make this highly probable. We have seen, however, that in spite of such a production of peroxide the organism is not killed. McLeod and Gordon have shown that anaerobes do not proliferate in media containing extremely small quantities of hydrogen peroxide (at concentrations of the order of two or three parts in a million) and have adduced this observation as evidence for the toxic nature of peroxide.

It is clear, on our views, that the effect of such small concentrations of peroxide will be similar to that of oxygen itself. It follows that, if this hydrogen peroxide be removed, after exposing the organism to it, and the correct reduction potential be set up, proliferation of the organism should occur. Nevertheless, we must expect that at some relatively high concentration, hydrogen peroxide will be actually toxic, *i.e.* its action will be irreversible, for at this concentration it will attack the substance of the organism to such an extent that death must ensue. The addition of any reducing substances can, then, have no reviving influence. Hence we may expect hydrogen peroxide to exhibit two critical concentrations—a lower one whose effect is reversible and which, acting like oxygen, increases the oxidation potential of the cell above the limiting point and thus prevents proliferation; and a higher one whose effect is irreversible and by directly oxidising the substance of the organism produces an irreversible condition; this may be regarded as the toxic or lethal concentration. Thus, between the two critical concentrations, we should be able to observe effects precisely similar to those obtained with oxygen and which only differ in their quantitative aspect. These expectations are fully borne out by experiment.

Exp. 8. A series of 11 tubes, *A*, *B*, ... *K*, was prepared so as to contain 10 cc. each of tryptic broth with concentrations of H_2O_2 varying from 0·03 % to 0·003 %. This was done by placing the appropriate volume of broth (9 cc., 9·1 cc.…9·9 cc.) in each tube, autoclaving the tubes and then adding the necessary volume of 0·3 % H_2O_2 (1 cc., 0·9 cc.…0·1 cc.) by means of a sterile pipette. These were prepared in duplicate so that finally there were two series of tubes containing diminishing quantities of H_2O_2, the highest concentration being 0·03 % in *A*, that in *J* being 0·003 % and that in *K* being zero. Each tube was then inoculated with three drops of a fresh culture of *B. sporogenes* in TB and incubated anaerobically at 37°. After 18 hours no growth was observed in any tube except in the two *K*'s where growth was abundant. So far McLeod and Gordon's observation of the growth inhibiting effect of very small quantities of hydrogen peroxide on the anaerobe is

confirmed, *i.e.* a concentration of 0·003 % H_2O_2 appears to be toxic. One series of the tubes was then marked *A'*, *B'*, ... *J'* and to each tube was added 1 cc. of neutralised sterile 10 % solution of cysteine hydrochloride. This amount of cysteine was sufficient to react with the highest concentration of H_2O_2 present in the tubes and to leave sufficient —SH over to give a strong nitroprusside reaction. The other series, *A*, *B*, ... *J*, was not disturbed. Both series were incubated anaerobically for a further 48 hours. The results are shown in Table IV.

Table IV.

Medium without added CSH ...	*A*	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*J*
Initial concentration of H_2O_2	·03 %	·027	·024	·021	·018	·015	·012	·009	·006	·003
Growth after further 48 hours ...	0	0	0	0	0	0	0	+ +	+ + +	+ + +
Medium with added CSH	*A'*	*B'*	*C'*	*D'*	*E'*	*F'*	*G'*	*H'*	*I'*	*J'*
Growth after further 48 hours ...	0	0	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +

The experiment shows (1) that the presence of very small quantities of H_2O_2 has an inhibiting influence on the growth of *B. sporogenes*; (2) that this inhibiting influence is similar to the effect of oxygen, for, on further incubation, growth occurs up to a tube which contained initially 0·009 % H_2O_2; (3) that the subsequent addition of cysteine makes growth possible in a tube which contained initially 0·024 % H_2O_2.

It follows that a concentration of H_2O_2 as high as 0·024 % is not lethal to all the organisms. Usually we have found no growth in *A'* or *B'* so that it would appear that the lethal concentration of H_2O_2 lies between 0·027 % and 0·024 %. On one occasion, however, we found a slight growth of the anaerobe in *A'*. The fact that growth occurs in *J*, *I*, *H*, where cysteine was not added, is due, doubtless, to the removal of the added H_2O_2 by the constituents of the broth, the organism then proceeding to create its limiting reduction potential in the manner we have described. The effect of cysteine is simply to accelerate the process of removal of H_2O_2 to a very marked extent and to create, itself, very quickly the necessary reduction potential for proliferation.

General Considerations.

The views we have put forward seem to us to provide a satisfactory interpretation not only of the results obtained by McLeod and Gordon [1925, 1, 2] in their experiments on chocolate agar, but of Callow's, of Novy's and of a variety of other observations on the growth of the strict anaerobes.

It is well known, for instance, that a large inoculation of *B. sporogenes* into glucose broth (and sometimes plain broth) will almost certainly give a good aerobic growth, whereas a small inoculation will give indecisive and

often negative results. It is easy to see why this must be, for with a large inoculation not only a large number of cells are introduced but an appreciable quantity of —SH from the inoculating fluid. This secures the necessary reduction potential for proliferation even in the presence of oxygen. If the quantity of —SH inoculated be small, the —SH may be oxidised before proliferation has commenced and hence the failure to grow. As stated earlier, the question becomes one of relative velocities; if once the organism can begin to proliferate and produce a stream of —SH compounds, or other substances of similar reducing nature, at a velocity greater than that at which they are removed by oxygen, aerobic growth will be possible. Catalase fails to secure aerobic growth simply because it cannot induce the correct reducing potential; its presence, however, protects the organism from the truly toxic irreversibly acting concentration of H_2O_2.

Since the velocity of oxidation is determined partly by the tension of oxygen it follows that different anaerobes will proliferate at different critical tensions of oxygen, these tensions being determined by the relative velocities of formation of substances responsible for the maintenance of the reduction potential and of their oxidation by oxygen. Similarly, different anaerobes will exhibit different susceptibilities to exposure to oxygen or hydrogen peroxide, the latent period in anaerobic growth being determined by the medium, the proteolytic activity (or power to produce reducing substances) and the value of the limiting reduction potential. We have confined our attention almost exclusively to *B. sporogenes*—a strongly proteolytic organism. With the highly saccharolytic and less proteolytic organisms it may be that —SH does not play so predominant a part and that the powerful activation of glucose, with consequent high reducing power, becomes the important factor.

When a substance does not give a reduction potential observable at the electrode it depends for its reducing power *in vivo* on its activation by a cell, or enzyme. Now formates are very highly activated by an organism such as *B. coli* and would, undoubtedly, have high reducing power in presence of this organism. It must not be concluded, however, that formates are "reducing" in presence of *any* organism. Our own observations indicate that formates are but feebly activated by *B. sporogenes* and in correlation with this we find that formates have very little or no effect compared with —SH.

An interesting point in connection with —SH is the effect of fresh tissues on the growth of the anaerobes. It is well known that such tissues are able to induce prolific growth, even aerobically, of most anaerobes. Now the effect may be due in part to respiration of the tissue, as Novy has pointed out, and it may be due, in part, to the presence of certain nutritionally valuable substances, but it is significant that with the animal tissues the latter not only contain a supply of —SH (in the form of glutathione) but a mechanism by which a certain supply can be maintained even aerobically [Hopkins, 1921; Hopkins and Dixon, 1922]. By this last circumstance alone an organism

such as *B. sporogenes* will find in an animal tissue its ideal *milieu*. Even the presence of autoclaved animal tissue will be extremely valuable in a medium which initially contains a little —SH or —S—S—, for the tissue still retains its power of producing —SH from —S—S— and hence of maintaining the supply of —SH.

Viability.

We have carried out many experiments to determine whether the presence of 0·1 % cysteine hydrochloride in a medium affects the viability of *B. sporogenes* and so far we have received, by repeated subculture into a medium containing 0·1 % cysteine hydrochloride, no indication that the substance has any harmful effect whatever on the organism.

Summary.

1. Neither oxygen nor hydrogen peroxide up to a concentration of 0·024 % is lethal to *B. sporogenes*.

2. *B. sporogenes*, after it has been exposed to oxygen or dilute concentrations of hydrogen peroxide, exhibits a long latent period in its anaerobic growth in tryptic broth.

3. This latent period is diminished or almost entirely removed by the presence of cysteine (or other —SH compound) in the medium.

4. The presence of a small amount of cysteine (or other —SH compound) in tryptic broth will induce good aerobic growth of *B. sporogenes*.

5. It is suggested that the latent period exhibited by oxygenated *B. sporogenes* is the time of incubation required for the inoculated non-proliferating cells of the organism to produce a certain minimum quantity of —SH (or other reducing bodies) from the medium.

6. This minimum quantity of —SH (or other reducing bodies) must be present in order to establish the limiting reduction potential which is necessary for proliferation of the organism. In a medium which maintains a higher (more oxidising) potential than this, proliferation of the anaerobe cannot occur. In a medium in which the cell is at or below this potential proliferation is possible.

7. It is shown that, on the assumption of the existence of a limiting reduction potential, which is necessary for the proliferation of an "obligate" anaerobe, all observations on the relationships of the anaerobes to oxygen can be satisfactorily interpreted.

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NOTE. (*Added November 1st*, 1926.) Whilst this communication was in the press the work of Hosoya, and Hosoya and Kishino (Scientific Reports from the Government Institute for Infectious Diseases. Tokyo, 1925, pp. 103 and 123) came to our notice. The Japanese investigators have established the following facts: that broth containing 0·001 % cysteine hydrochloride admits of rapid growth of various anaerobic bacilli and that —SH will induce the growth of strict anaerobes on a digest of gelatin which in the absence of the —SH is unsuitable for such growth. They have not, however, attacked the problem of the relationship of the anaerobes to oxygen in the manner developed in this paper; nor have they arrived at our conclusions.