CXXIII. OXIDATION-REDUCTION STUDIES IN RELATION TO BACTERIAL GROWTH.

II. A METHOD OF POISING THE OXIDATION- REDUCTION POTENTIAL OF BACTERIOLOGICAL CULTURE MEDIA.

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(Received June 30th, 1930.)

In the foregoing paper [Knight, 1930] the oxidation-reduction (O/R) potential of sterile meat broth was studied. It was shown that the potential of the broth slowly drifted to a definite level, depending on p_H , when oxygen was removed. It is obvious, therefore, that if it is desired to study the behaviour of a micro-organism under constant conditions of O/R potential of its surroundings, this can only be done at the final level of potential to which a broth will drift, unless a method can be devised whereby the potential can be held constant at any level.

In seeking some method of poisoning the O/R potential of a medium at a chosen level several possibilities at once present themselves. Thus, the presence of an O/R dye tends to poison the potential temporarily in the region of 50 % reduction [Cannan, Cohen and Clark, 1926]. The broth soon completes the reduction of the dye however, and the negative potential drift of the medium proceeds as before until it reaches its final level. The time during which the dye poisons the potential in the region of 50 % reduction can be prolonged by increasing the amount of dye, but there are obvious practical limits to this method. There is the further objection that the potential levels would be limited to the number of dyes and other O/R systems available. Also there might be a specific chemical effect of the dye on a particular micro-organism.

A second possibility would be to destroy or remove or completely oxidise the system responsible for the potential and then to poison at a chosen level by a suitable degree of reduction of an added O/R system. In the absence of oxygen and of any substance in the broth to react with the added system, the potential could then be held constant.

Attempts to oxidise completely the system by prolonged passage of oxygen have not been successful; neither have attempts to destroy or to remove it, without altering the nutrient properties of the medium.

¹ Working under a grant from the Medical Research Council.

A suitable synthetic medium to which a definite O/R system might be added is a further possibility, as yet unrealised.

It becomes apparent that static methods of poisoning in the presence of such a system as that in broth are at present unavailing, and that some dynamic method is necessary. That is, the negative drift of the medium must be counter-balanced by some opposing oxidising tendency. It is on these lines that the method to be described has been obtained.

Briefly, the negative potential drift is balanced by passing into the medium a controlled stream of very dilute oxygen in nitrogen. The concentration and rate of flow of the gas mixture are adjusted until there is equilibrium between the rate of reduction of the broth system and its rate of oxidation by the oxygen; the potential then remains constant.

The advantage of using oxygen lies in its comparative inertness as an oxidising agent, the ease and smoothness with which the rate of addition can be controlled, and the fact that no solvent etc. accumulates in the medium under examination.

METHOD.

To obtain a stream of very dilute oxygen advantage is taken of the small quantity of the gas (about 0.5 %) occurring in commercial cylinder nitrogen. In the previous paper a method for completely freeing the nitrogen from oxygen by passage over reduced copper gauze at red heat was described. For the present purpose the nitrogen gas-line to the electrode vessels is modified so as to by-pass a proportion of the stream of impure nitrogen before the copper gauze de-oxygenator (Fig. 1). Flowmeters (FM_1 and FM_2) are included in each branch of the gas-line so that the volume of gas passing through the purifier and that by-passed can be measured.

Fig. 1.

The manometer (M) measures the internal pressure at entry to the electrode vessels. It is used in controlling the gas supply to the individual electrode vessels. The by-pass to flowmeter FM_1 is a convenience to prevent a sudden

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rush of gas blowing over the liquid in the flowmeter on first opening the cylinder valve. It is afterwards closed.

A diagrammatic curve showing the stabilising of potential is given in Fig. 2 to which the letters in brackets refer. The technique which has been

Fig. 2. Diagrammatic curve illustrating the stabilisation of O/R potential of sterile broth (p_H 7.6) at E_h 0.00 volt.

found most satisfactory in practice is as follows. The electrode vessel and its contents are first de-aerated by a fairly vigorous stream of pure nitrogen with by-pass taps T and T' shut. The potential is allowed to drift negative to the value at which it is ultimately desired to poise it (A). Potential readings are made every half-hour. When the potential is sufficiently negative (B), the gas rate is reduced at the supply cylinder to give a suitable degree of stirring. The by-pass taps, T and T' , are then slowly opened to give a small flow of unpurified gas (C). Experience will tell what the by-pass flowmeter readings should be, for a given volume of medium, to give approximately the required potential level. Once the medium has been thoroughly de-aerated, and the electrodes have been in contact with it for several hours, small changes in the degree of reduction of the broth system are relatively rapidly recorded by the electrodes. With gradual alterations of the relative rates of purified and unpurified gas any desired potential level can be obtained with as great precision as the normal differences of the electrode readings will allow. When the gas rates are suitably adjusted potential readings are taken for another hour to make sure that equilibrium is attained (D). Provided the gas supply from the cylinder is adequate and the copper purifier is not exhausted, the apparatus may be left running for several days. The potential remains constant if the broth is sterile, or if there is no growth of the micro-organism being examined.

When the potential is constant, inoculation of spores or bacteria can be made (E) through a hole in the bung of the electrode vessel, normally plugged with a glass rod. If the volume of the inoculum is small, only a very slight temporary change of potential takes place (F), and the former level is regained

after about half an hour (*G*). Samples of the medium may be pipetted off similarly.

Poising in presence of oxidation-reduction dyes.

The general behaviour of electrodes in broth has been described. When the oxygen method of poisoning is used the agreement between electrodes is of the same order, namely, a difference of 10 mv. or less. In prolonged experiments, 12 hours or more, the agreement tends to become closer. Much greater precision can be given to the electrode readings, and the poisoning of the potential is made more refined, by the addition of small quantities of O/R dyes. A dye is chosen whose span of potential covers the desired level. The dye potentials then control the electrodes, and the latter agree to within 1 mv.

In this case the dye acts as an "indicator" of the potential of the broth system and the potential of the latter is recorded at the electrode *via* the dye potential.

In any particular case it must be shown that there is no specific action by the dye. It is possible, for instance, that a dye might be toxic to a particular organism. In the absence of any specific effect, the addition of small quantities of dye has the further advantage that, with care, a simple potentiometer circuit can be used in the measurement of potential level, at least in orientating experiments.

Span of potential.

Commercial cylinder nitrogen contains about 0.5 % oxygen. Using 0 % to 100 % of unpurified gas mixed with oxygen-free nitrogen the range of potentials between the lowest value to which a medium will drift and a potential of about $E_h + 0.175$ volt at p_H 7, can be covered by suitable alteration of proportions of the two gases and of the rate of flow through the electrode vessel. The positive limit at which it is possible to work with these gas mixtures is rather indefinite, but is more positive the lower the p_H . This is to be expected from the general slope of the E_h - p_H curve [Knight, 1930, Fig. 2].

It may be necessary to use a small flow of air (from a cylinder of the compressed gas) instead of by-passing unpurified nitrogen, if it is desired to poise in a zone positive to about $E_h + 0.25$ volt.

The electrodes do not behave well positive to a zone at about $E_h + 0.175$ volt, in the absence of O/R dyes. By analogy with the behaviour of electrodes in O/R dye solutions, this is probably due to the almost complete oxidation of the systems responsible for the broth potentials. In the presence of a suitable dye or other reversible system it would be possible to work at high levels with as great ease as at the lower levels.

No attempt has been made to correlate actual partial pressures of oxygen and velocities of flow with the O/R potential obtained in any sample of medium, although in bacteriological literature it is customary to correlate "degree of anaerobiosis" with partial pressure of oxygen. If O/R potential is taken as the index of degree of anaerobiosis there is no necessary relation

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between oxygen pressure and bacterial growth. The pressure of oxygen capable of being tolerated by an anaerobe will vary with the reducing intensity and capacity of the medium.

While the above method of poisoning has at present only been used with bacteriological culture media, it could obviously be applied to any other system which exhibited a negative potential drift with time, for example, cell and tissue suspensions.

SUMMARY.

A method has been described whereby the oxidation-reduction potential of bacteriological culture media can be held constant.

The expenses of this investigation were defrayed by the Government Grants Committee of the Royal Society and the Yarrow Research Fund of the London Hospital.

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