VII. ON THE BEHAVIOUR OF "INDIFFERENT" ELECTRODES WHEN USED FOR THE DETERMINATION OF OXIDATION-REDUCTION POTENTIALS IN THE PRESENCE OF HYDROGEN.

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IN some observations on the reduction potential of cultures of anaerobic organisms recently reported [Lepper and Martin, 1930] we found that gold electrodes gave entirely different results from iridium electrodes if hydrogen was liberated during growth. The gold electrode remained poised by the feeble parahaematin-haemochromogen oxidation-reduction system present in the muscle fibres of the meat used in the medium. The iridium electrodes, on the other hand, were rapidly converted into hydrogen electrodes and reached the potential of the buffer solution surrounding the meat. In order to get a better understanding of the behaviour of these electrodes we have examined their potential in weakly poised and unpoised buffer solutions in the presence of nitrogen, hydrogen and oxygen.

Preparation of the electrodes.

The iridium electrodes were prepared by the method of Westhaver [1905], the glass being given two coats of iridium.

The gold electrodes were made by heavily gilding platinum plates electrolytically in a bath of gold cyanide. As soon as the platinum appeared to be well gilded the electrode was removed from the bath, dried and heated in the blowpipe. This process was repeated four or five times until the electrode was a deep gold colour after heating. By this means a substantial film of gold was alloyed on to the platinum before it was finally coated with electrolytically deposited gold. Electrodes made in this way were much less sensitive to hydrogen and oxygen than merely gilded platinum electrodes.

Experiments in weakly poised solutions.

The electrodes were placed in phosphate mixtures containing indigo-carmin of different molecular strengths. The air was expelled from the electrode vessel by nitrogen deprived of traces of oxygen by passing through a

solution of anthraquinol made by reducing an alkaline solution of anthraquinone with sodium hydrosulphite [Conant and Fieser, 1924]. To secure fine bubbles the gas was made to traverse a piece of kieselguhr filter candle as shown in Fig. 1. When the air had been displaced from the phosphate solution a few drops of weak sodium hydrosulphite were added from a burette to reduce the indigo-carmin in part and readings of the potential were taken until it had become steady. Hydrogen free from oxygen was now run through the apparatus until the change in potential of the electrodes which took place had again reached its limit.

When the concentration of indigo-carmin was $0.001 M$ the gold electrode remained perfectly stable on substituting hydrogen for nitrogen, but in a concentration of $0.0001 M$ the potential rose 8 mv.

With a concentration of $0.002 M$ indigo-carmin the iridium electrode showed a rise of 10 mv. and with a concentration of $0.0001 M$ the electrode had nearly reached the potential of the hydrogen electrode by the end of half an hour. The behaviour of the iridium electrode is shown in Fig. 2.

Fig. 1. Wash bottle for removal of oxygen from nitrogen and hydrogen.

Fig. 2. The effect of hydrogen on an iridium electrode in different concentrations of indigo-carmin.

Experiments with unpoised buffer solutions.

A phosphate mixture was placed in the electrode vessel and the air was expelled by nitrogen. When sufficient time had been given to remove all the

oxygen from the solution hydrogen was substituted. At the end of $1\frac{1}{2}$ hours the negative drift of the gold electrode was 45 mv., whereas the iridium electrode had reached the potential corresponding to the hydrogen ion concentration of the solution, -0.7155 v., where it remained steady (Fig. 3).

Fig. 3. The effect of hydrogen and oxygen on gold and iridium electrodes in phosphate buffer p_H 8.0.

..... Iridium.

○—○—○ Gold.

The hydrogen was then displaced by nitrogen. After 45 minutes the gold showed a potential increase of 16 mv. and the iridium had fallen but 28 mv., indicating that the hydrogen absorbed by the iridium electrode is given off to an atmosphere of nitrogen only very slowly.

Lastly, when oxygen was run through the solution a rapid fall of potential occurred with both electrodes. At the end of 30 minutes the gold electrode again gave a steady potential. The iridium, on the other hand, after the first sudden fall to $+0.1$ v. continued to show a slow positive drift throughout the 5-hour period of observation.

The maximum potential of -0.125 v. observed at the gold electrode during the second period with nitrogen probably represents closely the potential of a really indifferent electrode at this p_H , as the oxygen present in the electrode at the time of immersion would, we presume, have been removed by the hydrogen.

It can be seen from Fig. 3 that gold appears to be much more sensitive to oxygen than it is to hydrogen.

DISCUSSION.

The explanation of the different behaviour of electrodes of gold and iridium during the growth of microbes when hydrogen is evolved appears to be that, the evolution of hydrogen being occasioned by an irreversible process and gold being relatively insensible to hydrogen, there is no reversible oxidation-reduction system present with a more negative range than the parahaematin-haemochromogen with which the gold electrode can come into electro-chemical equilibrium.

This interpretation is supported by the fact that when the more electro-negative dye safranine was added to the culture medium so as to make a concentration of about 0.01 %, in 1 hour after inoculation with *B. welchii* the E.M.F. afforded by the iridium electrode fell as before to that of the hydrogen electrode, but the potential of the gold electrode, instead of remaining poised by the parahaematin-haemochromogen dropped steeply until it was poised by the safranine around -0.33 v.

Fig. 4. The behaviour of gold and iridium electrodes when *B. welchii* grows in meat medium.
 Curve 1. Iridium in meat. Curve 2. Iridium in meat containing safranine.
 Curve 3. Gold in meat. Curve 4. Gold in meat containing safranine.

The iridium electrode behaves otherwise, because it becomes charged with hydrogen from the solution and functions as a hydrogen electrode. The reason for the hydrogen overvoltage noticed by Gillespie [1920] and also by Cannan, Cohen and Clark [1926] which also occurred in some of our experiments, is not obvious. The gas evolved contains less than 50 % hydrogen, but the conditions under which it is formed are conducive to supersaturation of the solution. Moreover, it is atomic hydrogen which is first separated, and this may find direct access to the electrode.

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