THE EFFECT OF SUNLIGHT AND TEMPERATURE ON THE REDOX POTENTIAL OF LIQUID MEDIA

BY

S. ZADOR*

From the Scientific Services, Snowy Mountains Authority, Cooma, New South Wales, Australia

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It is often found that the characteristic patterns obtained from redox potential measurements in metabolic studies are invalidated by possible variations in the media as well. Consequently the patterns so obtained do not give a true picture of the metabolism, and therefore do not reflect properly the manifestations of life.

Experiments were carried out to show the extent of these variations, and the methods and results of this work are detailed below.

Methods

Measurements of redox potentials were taken with the aid of a Jones pH meter. The cells used were *Bact. coli*. The medium was McConkey's single broth. The organisms were incubated at 37° C. for 36 to 48 hours. Two experiments were run simultaneously, one with organisms, referred to as "experimental," the other without them, referred to as "control."

For each potential reading the pH was also determined by a rough method, using indicator paper of the appropriate range. The pH, as is known, influences the redox potentials. However, according to Hewitt (1950), the introduction of a buffer solution would considerably complicate the situation by bringing a number of unknown factors into the picture.

Results

The readings were plotted in a co-ordinate system, millivolt readings on the ordinate and time on the abscissa. Seven graphs are presented.

* Present address: West London Hospital Medical School, London, W.6.

Fig. 1 shows the variations in two sterile liquid media (McConkey's single broth), one exposed to daylight and temperature changes (Curve No. 1), the other (Curve No. 2) kept in the dark. One can see the variations in the first part of the redox curve as compared with the slight variations in the one kept in darkness. The temperature was on average 10° C. higher in the exposed one, which also indicates that a considerable amount of heat reached the media (the test-tube was kept at the window). In the exposed media the decrease is more accentuated. This decrease is perhaps due to the beginning of decomposition of the sugar in the media at a considerably higher temperature than the one kept in the dark; the oxidation process may also be due to this higher temperature. The oxidation of the sugar is affected and possibly initiated by the daylight, and the temperature would only have a secondary effect within the range of these temperature variations.

In Fig. 2 the curve marked as "light int." shows the effect of sunshine through the glass in the window, beaker, and test-tube. In this filter system some ultra-violet rays were absorbed, and the heat waves penetrated. The temperature of the media was higher than in the one marked "light external." In this latter one the test-tube was placed outside the laboratory.

FIG. 3.—Redox potentials in lactose broth in the dark at 37° C.

FIG. 4.—Redox potentials in glucose broth in the dark at 37° C.

FIG. 5.—Redox potentials in mannitol broth in the dark at 37° C.

The least variation is detected in the medium kept in the dark at a relatively low temperature compared with the curves "light internal" and "light external." Also, when the curves are inspected after a night (14 hours) the tubes exposed to light present variations while the ones kept in the dark are practically unaltered. Variations in all experimental curves in the first two hours are probably due to the temperature differences to which the media are subjected when taken out of the refrigerator.

Figs. 3-5 represent experiments in which the inoculated experimental tubes and the sterile controls were kept in the dark at 37° C. In Fig. 3 the medium was lactose broth and it will be seen that the experimental curve falls steeply in 12 hours, while the control remains more or less steady, hence this is a genuine fall. After that, until 26 hours, the "experimental" hardly changes, while the "control" shows a steady increase. Had the control stood steady, the experimental curve would probably have decreased even more. On the other hand, the increase of the experimental curve between 26 and 34 hours would have been further increased had it not been counteracted by the fair decline of the control curve. The declining slope of the experimental curve between 34 and 48 hours can be considered as a true decline, i.e., the corresponding section of the control curve does not show any variation. However, the decreasing slope of "experimental" in this section does not signify growth since the low *pH* attained in the phase between 12 and 26 hours would not provide the optimum milieu for further growth activities. Also the redox potential of this phase is already fairly low for growing aerobic organisms.

Figs. 4 and 5 show the changes obtained with glucose and mannitol broth. In both cases the changes in the control tube are so great that they interfere significantly with the interpretation of the experimental curves.

Figs. 6 and 7 give the corrected experimental curves of Figs. 4 and 5 respectively, demonstrating rather convincingly how much interference can be attributed to the redox variations in the sterile media and how misleading the interpretation of

FIG. 6.—Experimental curve of Fig. 4 corrected for changes in medium.

FIG. 7.—Experimental curve of Fig. 5 corrected for changes in medium.

the experimental curves could be if the changes in the media proper were not borne in mind.

Discussion

Proteins are able to carry oxygen by the presence of their SH_2 groups. Sugars in the somewhat increased temperature of the incubator (37°C .) slowly start to oxidize, give off hydrogen, and, by combining with the oxygen, form H_2O_2 . When sunshine is present, the formation of H_2O_2 is speeded up. The ultra-violet rays of the sunshine help to form H_2O_2 ; its concentration depends on the wavelengths and the period of exposure. While the sugar is oxidized, the oxygen, by taking up the liberated hydrogen, is reduced. The presence of this free reduced substance increases the redox potential. This is marked by a steep increase in the positive direction on the graphs. When these substances are protected against sunshine (kept in the dark) the variation in redox potential becomes practically negligible. The substances mentioned above are all present in the common culture media, hence they are likely

to produce these reactions and, in so doing, a fairly irregular curve on the redox graphs and considerably interfere with the shape of the experimental curves. In other words, when redox curves are read, the changes produced by the media themselves have to be interpolated in order to be able to reconstruct and obtain the "real" curve and draw the proper conclusions.

Summary

It is found that sterile culture media undergo changes when exposed to alterations in light and temperature. These changes cause considerable variations in the redox curves of the sterile media used as controls. The possible cause of these changes is discussed. Their importance in avoiding misinterpretation of experimental results and drawing false conclusions is pointed out.

REFERENCE

- Hewitt, L. F. (1950). *Oxidation-Reduction Potentials in Bacteriology and Biochemistry*, 6th ed., p. 107. Chapter on the electrode potentials observed in bacterial cultures. E. and S. Livingstone, Edinburgh and London.