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Factors Affecting Oxidation-Reduction Potentials Produced by Bacteria in Synthetic Media.

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Measurements of the apparent oxidation-reduction potentials established in a synthetic medium by the growth of *Escherichia coli* showed that many irregularities were encountered which were not present when determinations were made in peptone nutrient broth cultures of the same organism. It is the object of this paper to present a study of the factors involved in these irregularities. The elimination of such would make it possible to obtain consistent data from which valuable conclusions might be deduced.

A vacuum tube null-point instrument drawing a maximum current of 10^{-11} ampere was utilized in making the physical measurements. No polarization phenomena were observed and the apparatus gave calculated values for easily polarizable half cells. Bright platinum electrodes were used. A liquid medium made by weighing the requisite C.P. or analytical reagents and adding to redistilled water allowed the test organism, *Escherichia coli,* to grow vigorously. This basic solution, a slight modification of that of Koser and Saunders,[1] was composed of Na_2HPO_4 1.4 g, KH_2PO_4 1.0 g, NaCl 2.0 g, $MgSO_4$ (anhy.) 0.1 g, l-asparagine 3.0 g, l-tryptophane 0.2 g, d-glucose 2.0 g, and redistilled water 1 liter. Sterilization was accomplished by filtration. The final pH was 6.9. A nutrient broth medium used for comparison was composed of beef extract (Swift) 3.0 g, peptone (Difco) 10.0 g, NaCl 5.0 g, and redistilled water 500 ml. This was adjusted to a pH of 6.9, sterilized in the autoclave, and to it was added 500 ml of sterile phosphate buffer (pH 6.9 at 37.5 C). The buffer was present in a final concentration of M/30.

Culture vessels consisted of 180 ml electrolytic beakers stoppered with rubber stoppers containing appropriate holes for the electrodes, for a stirring apparatus, for the agar-KCl bridge and to allow for gas exchange. The medium, previously inoculated with *E. coli,* was pipetted into the electrode containing vessels after the latter had been sterilized in the autoclave. Vessels were incubated in a water bath at 37.5C±0.02C.

The basic synthetic medium gave very irregular results when

[1] Koser, S. A., and Saunders, F., *J. Infect. Dis.,* 1935, **56**, 305.

incubated under stationary conditions. Irregularities appeared between the values obtained for like duplicate vessels and between duplicate electrodes in the same vessel. The Eh intensity levels in the synthetic medium when read by means of platinum gauze, foil, or wire electrodes were dependent upon the type of electrode used. This is demonstrated in Fig. 1. These phenomena, apparently not due to acid production, did not appear in broth media.

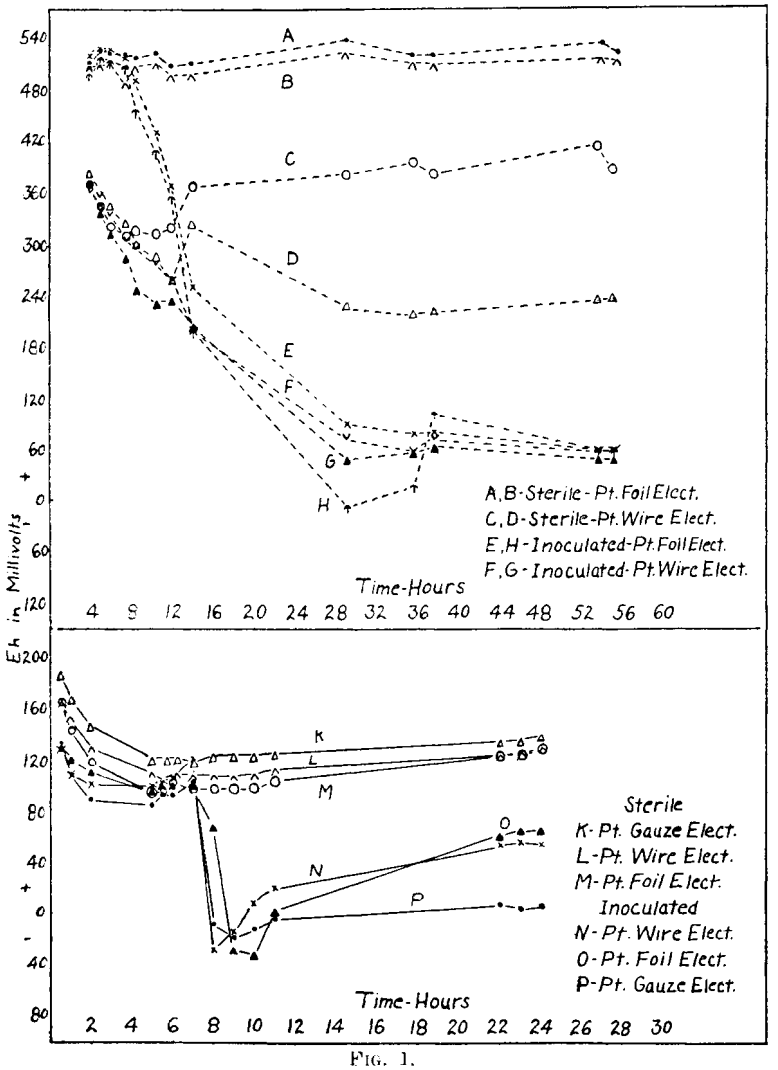

FIG. 1.

Time-potential curves obtained in the basic synthetic medium under aerobic (- - - -) and anaerobic (——) conditions. Each curve represents an average of the readings obtained from duplicate electrodes in the same vessel.

When purified nitrogen was bubbled through cultures of the basic medium irregularities were absent for the first seven hours of incubation (lower portion of Fig. 1).

Thus it would appear that the potentials that have ordinarily been attributed to the production of measurable oxidation-reduction systems by growing organisms are to a certain extent dependent upon the presence of poising materials present in the medium before growth is initiated. Such a poising agent can act as a mediator between the bacteria and their systems and the electrodes. Preliminary experiments have supported this concept. Stratification of growth has been shown to occur in some media and it seems that the more comparable results obtained by bubbling nitrogen or other gases through a medium might in part be due to agitation in addition to the removal of oxygen electrode effects. Simple mechanical agitation introduces complications because of the oxygen lability of the systems present.

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Antisera for Organ-Proteins.

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In none of the experimental work conducted in this laboratory could tissue-proteins be completely freed from blood proteins, nor has it been found possible to separate the blood proteins quantitatively from the autolyzed tissue-protein. So, antisera to tissue-protein or to autolysates always contained precipitins for blood proteins. Absorption *in vitro* with blood proteins usually removed not only their precipitins but also any precipitins that may have been formed for tissue-proteins. One investigator, Gilman,[1] working with urinary protein from a patient with severe chronic nephritis, was able to remove quantitatively the precipitins for hemoglobin, pseudoglobulin, and albumin by absorption *in vitro,* and obtained a precipitin that was specific for renal protein as found in an autolysate of the kidney When the renal autolysate was used for immunization, absorption *in vitro* of the blood-protein precipitins removed all of the precipitins.

[1] Gilman, G., *J. Urology*, 1935, **34**, 727.