studies were made on the high-boiling fractions. Neither the acid nor the basic washes contained appreciable quantities of C^{14}-labeled compounds.

It is apparent that $C^{14}Cl_4$ of low specific activity can be produced by the recoil method.

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Double-Gradient Agar Plates

A considerable portion of the *in vitro* study of the action of antimicrobial compounds consists of the investigation of possible effects of inorganic or organic substances on the potency of the compounds. Such investigations are usually performed with only a single or a few concentrations of each substance and antimicrobial compound. To obtain complete information concerning possible suppressing or enhancing effects, or both, of a test substance on a compound, however, a large number of substance-compound ratios should be screened. Correspondingly large numbers of flasks, tubes, or petri plates are required for such studies if conventional techniques are employed.

A useful and widely adopted method for the establishment of numerous gradually differing concentrations of an antimicrobial compound (or, alternately, of an essential nutrilite) in a single petri plate has been described by Szybalski (*1*). His method may be designated the single-gradient plate technique. Sacks (*2*) has described recently a type of double-gradient plate in which K_2HPO_4 is contained in the medium in the first or lower layer (which is solidified while the plate is in an inclined position) and KH_2PO_4 in the second or upper layer of medium (solidified while the plate is in a level position). Thus a continuous change in the ratio of the phosphates is established across the horizontal axis of the medium, with a resulting series of *p*H reactions from 5.6 to 7.8 over the surface of the agar. Such a plate can be used to determine the influence of *p*H on (i) growth, virus propagation, or other physiological activities of microorganisms and on (ii) the potency of an antimicrobial agent or the utilization of a nutrilite, provided that an appropriate concentration of the particular compound is incorporated in a thin (third) layer that is spread evenly over the surface of the agar medium.

This communication (*3*) describes the inclusion of a test substance (a metallic salt) in the first layer and an antimicrobial compound in the second layer of nutrient agar gradient plates. Thus a continuously graded ratio of test substance/compound is established over the surface of the medium. Preliminary titrations with single-gradient plates are used for determination of the concentrations of test substance or compound that completely inhibit, partially inhibit, or fail to inhibit the microorganisms. In the actual tests, the appropriate single-gradient plates are included to serve as controls. The test organisms are spread evenly over the surfaces of the control and experimental plates; our most consistent results are obtained with an inoculum per plate of not more than 10^3 viable cells contained in 0.05 ml of nutrient broth. An alternate method of inoculation consists of the streaking of cells of several test strains at right angles to the double gradient.

The observations made with the double-gradient plates clearly indicate instances of suppression or enhancement of an antimicrobial compound by a test substance. A diagrammatic representation of some of the results obtained with a strain of *Pseudomonas aeruginosa* is given in Fig. 1. It may be observed that Fe^{++} but not Cu^{++} suppresses the antibacterial activity of oxytetracycline and that the toxicity of neither of the ions is affected by the drug. In contrast, the antibacterial activity of kojic acid is affected by both Fe^{++} and Cu^{++}; certain proportions of metallic ion/compound are more toxic than either the ions or the compound alone, and other proportions are less antibacterial than the compound itself. As with oxytetracycline, however, the toxicity of the metallic ions is not suppressed by kojic acid. These observations are in agreement with results obtained previously with *Pseudomonas* by other methods (*4*).

It is believed that use of the double-gradient plate method can facilitate research not only in studies of antimicrobial compounds, but also in certain areas of microbial nutrition, physiology, and virology.

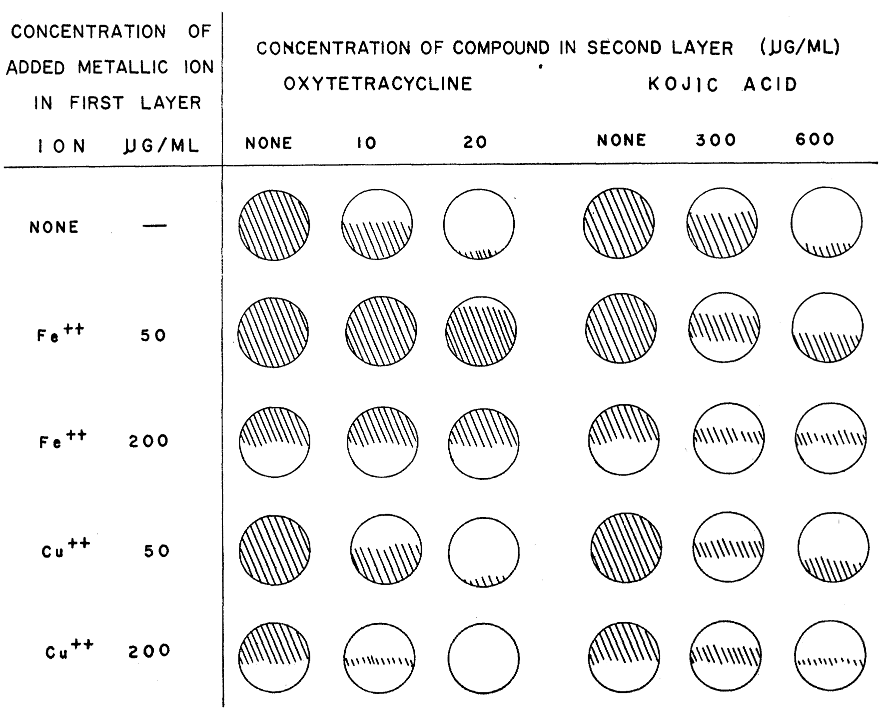

Fig. 1. Extent of bacterial growth on double-gradient plates containing varying quantities of ferrous or cupric sulfate and either oxytetracycline or kojic acid. The shaded areas represent visible growth of *Pseudomonas aeruginosa* after 18 hours at 37°C.

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