and backs are: proven useful and recognition Organ. 28:327, is small, nonselective cholera the shown cases and out, cholera vibrios. (Finkelstein was antigen of 757-63 was agglu-
tinated to the titer of Z49 antigen and reduced the titer of that antigen for the homologous bacteria from 1:6,400 to 1:200.

Thus, the antigenic composition of culture 757-63 was determined to be 3,10:Z49, z10:Z49, 1,5. Since it was possible to derive a culture indistinguishable from S. lexington (3,10:Z49;1,5) from culture 757-63, it was regarded as a complex form of that serotype, and comparable to the complex culture of S. infantis (6,7:Z49;1,5) cited above. In complex cultures which possess a common major antigenic component in both phases, it usually is not possible to recover that component after it has been suppressed by passage through appropriate antisera. However, in the culture described here it was possible in one instance to recover the Z49 antigen. Similar recoveries have been noted in the case of S. goerlitz (LeMinor and Edwards, Ann. Inst. Pasteur 99:469, 1960), S. simonsbury (Edwards, Moran, and Bruner, Proc. Soc. Exptl. Biol. Med. 66:230, 1947), and in an unnamed serotype having the antigenic formula 6;8:d;i:- (Edwards, Moran, and Bruner, J. Immunol. 60:529, 1948).

POLYMIXIN AGAR AS AN ADJUNCT IN THE ISOLATION OF EL TOR VIBRIOS

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El Tor cholera has recently occurred in epidemic form in much of Southeast Asia and the islands of the Western Pacific. The large numbers of cases and the attendant extensive epidemiological surveys placed a heavy burden on local laboratories. Many were poorly equipped to carry out, in large scale, elaborate procedures for isolation and identification, especially those requiring expensive or unusual chemicals. Recently, a simple, nonselective nutrient agar medium was shown to be satisfactory for isolation of El Tor vibrios (Finkelstein and Gomez, Bull. World Health Organ. 28:327, 1963) when coupled with the oblique-light technique advocated by Lankford (J. Microbiol. Soc. Thailand 3:10, 1959) for recognition of the characteristic colonies of cholera vibrios. Similar observations were noted by Feeley (J. Bacteriol. 84:606, 1962) with classical cholera vibrios. Although the technique has proven useful and practical, two potential drawbacks are: (i) that a certain element of judgment and ability is needed to recognize and pick the characteristic cholera colonies among those of the commensals which may also be present, and (ii) in a small percentage of specimens overgrowth by commensals may interfere. The latter problem is minimal in specimens from typical early cholera cases but could be of greater consequence in the case of carriers passing only a small proportion of vibrios relative to their normal enteric flora. The sensitivity of the technique could be enhanced if growth of the indigenous normal flora could be reduced.

Recently, Han and Khie (Am. J. Hyg. 77:184, 1963) reported that El Tor vibrios, in contrast to classical cholera vibrios, were resistant to polymyxin (PM). Since PM has activity against many intestinal microorganisms (Schwartz et al., Antibiot. Ann. 1959-60, p. 41, 1960), we considered that it might prove advantageous to employ PM as a selective agent in the procedure for isolation of El Tor vibrios.

A total of 66 strains of El Tor vibrios (including isolates from the Philippines, Thailand, Korea, Malaya, New Guinea, Indonesia, Burma, and Calcutta) were employed in the present study.
Most were recent isolates, although some older strains from the Middle East and Celebes were included. Eight strains of classical cholera vibrios were also tested. The array of cultures included some nonhemolytic strains of El Tor vibrios which can be identified as such by other means (Finkelstein and Mukerjee, Proc. Soc. Exptl. Biol. Med. 112:355, 1963; Mukerjee, Bull. World Health Organ. 28:33, 1963; de Moor, Trop. Geograph. Med. 15:97, 1963; Finkelstein, Nature, in press).

In confirmation of the report of Han and Khie, all of the El Tor strains possessed a high degree of tolerance for PM (Polymyxin B sulfate, Chas. Pfizer & Co., Inc., Brooklyn, N.Y.) as revealed by tests in which meat extract-agar plates containing graded concentrations of antibiotic were streaked with cotton-tipped applicators which had been dipped in 1:100 dilution of broth culture. Growth of the El Tor strains was undiminished at 10 units of PM per ml of agar medium, although partial inhibition of growth of occasional strains was noted at PM levels of 30 and 100 units. El Tor strains cultivated on the 10-unit PM agar retained their capacity to react with specific antisera in slide agglutination tests. The six strains which were tested at a higher PM level, 300 units, were completely inhibited. In contrast, growth of classical cholera vibrios was markedly or completely inhibited by 10 units of PM but not appreciably affected by 3 units. More sensitive tests of plating efficiency of El Tor cultures indicated that recovery was complete on agar containing 10 units or less of PM per ml; on media containing 30 units of PM per ml, the number of colonies was reduced. Growth of the aerobic flora of normal human stools was virtually completely inhibited at the 10-unit level of drug.

El Tor strains were somewhat more sensitive to PM in alkaline (pH 8.5) peptone broth in viable count growth curve studies employing inocula of 10⁵ vibrios per ml. Under these conditions, some inhibition of growth was manifest at PM concentrations of 3 units or more per ml of broth, but not at 1 unit.

To determine whether addition of PM would enhance the sensitivity of the isolation procedure, samples of serial dilutions of an El Tor strain were added, in parallel, to peptone broth, with and without 1 unit of PM, which was contaminated by the addition of suspended normal human stool. PM (10 units per ml) plates streaked after 6 hr of incubation of the peptone broth cultures yielded El Tor colonies in pure culture from tubes which had been inoculated with as few as five to seven vibrios per ml initially. On drug-free agar, recognition of El Tor colonies among those of the commensals was possible, by the oblique-light technique, only when the inoculum was 100 times greater. Inclusion of PM in the peptone broth did not appear to have any effect.

Ten normal stool samples were suspended in tubes of peptone broth which were then seeded with ten strains of El Tor vibrios at a level of approximately 10⁴ per ml. The El Tor vibrios were recovered in practically pure culture on PM agar streaked after 6 hr of incubation in every instance. Growth of commensal colonies when it occurred on PM agar was restricted to minute colonies which could not be mistaken for El Tor colonies. On the other hand, on the drug-free medium streaked in parallel, although recognition of El Tor colonies was possible by the oblique-light method in each instance, in many cases the commensal colonies were estimated to be greater than 99.9% of the total growth.

On the basis of these observations, it is concluded that the use of PM agar may facilitate the recognition of El Tor vibrio colonies and enhance the sensitivity of the isolation techniques described previously. This modification, it should be stressed, cannot be used for isolation of classical (non-El Tor) cholera vibrios, although it might be useful in some instances for the differentiation of the two groups of agglutinable vibrios which are responsible for epidemic cholera or for the selection of El Tor vibrios from mixtures with the classical variety.