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Motility in the Examination for B. Typhosus and Related Pathogens.

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(Introduced by Lloyd Arnold.)

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Bacteriologic diagnosis of typhoid fever and related infections has centered about the utilization of biochemical differences between the pathogenic organisms concerned and the non-pathogenic forms associated with them. The usual initial procedure in the examination of a suspected typhoid stool is seeding upon some lactose plating medium to eliminate all but the non-fermenting organisms present. The principal reasons to explain the failure to isolate the typhoid bacillus are: scarcity of *B. typhosus* in the stool, or the overwhelming antagonistic action of the associated bacteria. Glycerin, bile, and selectively bacteriostatic substances such as brilliant-green have been incorporated into the stool fluid or into the media. The object is either to forestall the destruction of whatever typhoid bacilli may be present or to hinder the growth of the non-pathogenic organisms of the mixture without inhibiting *B. typhosus*.

Given a mixture in which the desired bacteria were present in very small numbers, it would seem, *a priori*, that instead of inhibitive methods, enrichment would be the procedure of choice. Based upon the conception that, under similar conditions, *B. typhosus* and the Salmonella organisms are more actively motile than *B. coli*, the principal interfering organism, we have experimented with methods of selective enrichment.[1]

Our first attempts with liquid media were only partially successful. Difficulties with diffusion and convection currents during incubation were such as to make the results very irregular. The next trials were with semi-fluid agar in test-tubes; the inoculum was introduced into the bottom of the column of soft agar by a capillary pipette. Disruption of the agar by the pipette allowed rapid ascent of all the organisms in the test mixtures or in feces. Our best results have been with small U-tubes containing ordinary beef infusion to which 0.2% agar has been added. These U-tubes are made of soft glass tubing of an internal diameter of 8 mm. and a

[1] See also: Wassen, Anders, *C. R. de la Soc. de biol.*, 1930, **104**, 523.

thickness of 1 mm. The tubing is cut into 15 cm. lengths and bent into U-tubes after heating over a fish-tail burner. Melted agar is poured into the U-tubes until the column of medium is 4 cm. long. Cotton plugs are put into both ends of the tube, and the outfit is autoclaved. About 0.1 cc. of fecal suspension is placed upon the surface of one end of the agar column, the tube is incubated for 18 hours at 37°C. and then Endo or eosin-methylene blue plates are inoculated with agar removed from the opposite side of the U-tube by means of a sterile wire loop. The more actively motile typhoid and paratyphoid organisms are usually obtainable in predominating numbers and frequently in pure culture. It has been found that the optimum amount of medium for an 18-hour incubation period is a column 4 cm. in length. Evaporation is rapid in these tubes, so that outfits prepared in advance and stored in the ice-box should be examined carefully to insure the presence of an adequate amount of

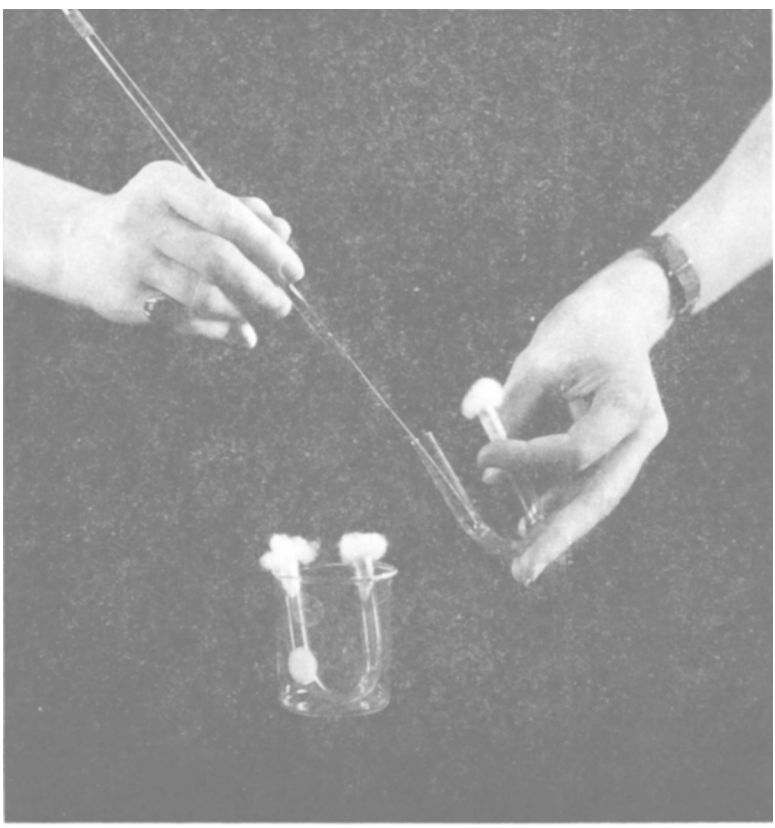

FIG. 1.

medium. Limited application of this procedure to routine fecal specimens has given excellent results and has led in one case to the isolation of *B. paratyphosus B.*, which did not appear on the plates made directly from the specimen.

The table illustrates typical results obtained with dilutions of *B. typhosus* made in a 24-hour broth culture of *B. coli* and plated on Endo by means of glass spreaders.

TABLE I.

Tube	Dilution of *B. typhosus*	Detectable Colonies of *B. typhosus.*	
		Direct plating	From U-tube
1	10^{-1}	29	85
2	10^{-2}	3	all
3	10^{-3}	1	95
4	10^{-4}	2	100
5	10^{-5}	1	90
6	10^{-6}	0	**80**
7	10^{-7}	0	90
8	10^{-8}	0	0

Tubes 6 and 7 gave no colonies on direct plating, but after growing through the U-tube presented the same result as the lower dilutions. The 1:100,000,000 dilution apparently contained no typhoid bacilli. This method is not offered to take the place of the usual plating of fecal specimens, but to supplement it, and it is, of course, not applicable to the dysentery group of bacilli.