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Use of Deoxycholate in Separation of In Vivo Growing *Salmonella typhimurium* from Tissue Debris

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To study the characteristics of bacteria grown in vivo (H. Smith, p. 1–24, *in* Microbial behaviour *in vivo* and *in vitro*, Cambridge Univ. Press, London, 1964), it is desirable to obtain them free from tissue components. Salmonellae are primarily located intracellularly in the host (E. Suter, Bacteriol. Rev. **20**:94, 1956) which complicates their separation from tissues, a problem which J. H. Hanks (J. Bacteriol. **62**:521, 1951) encountered with *Mycobacterium lepraemurium*. The primary difficulty lies in separating them from the mitochondria present in homogenates of spleen and liver. Experiments employing differential centrifugation of homogenates of organs obtained from infected, moribund mice, with repeated alternate cycles of 167 × *g* and 2,400 × *g*, yielded preparations still grossly contaminated by tissue debris as viewed under phase microscopy. Observation of fractions obtained after centrifugation, at 2,400 × *g* for 90 min, in a 10 to 70% sucrose gradient, revealed that the bacteria-rich layers (55 to 67.5% sucrose) were turbid because of the presence of numerous mitochondria. Since sodium deoxycholate (DOC) is known to lyse mitochondria (M. C. Watson and P. Siekevitz, J. Biophys. Biochem. Cytol. **2**:639, 1956) and does not affect *Salmonella* (E. Leifson, J. Pathol. Bacteriol. **40**:581, 1935), it was chosen to treat the homogenates. Exposure of homogenates to concentrations of DOC up to 0.3% in distilled water for 1 hr at 5 C and at *p*H values from 6.0 to 7.6 showed no effect on bacterial viability, yet resulted in mitochondrial lysis. The final procedure adopted, to rid the bacteria of tissue debris, was as follows. All manipulations were conducted at 0 to 5 C to prevent multiplication of the bacteria. Livers or spleens were homogenized with an equal volume of water in a VirTis homogenizer in an ice bath for 3 min at 29,000 rev/min. Homogenate was diluted 1:25 (w/v) with 0.3% DOC, incubated at 5 C for 30 min, and then centrifuged at 16,000 × *g* in the high-speed head of an International PR-2 centrifuge. The pellet, resuspended in a small amount of 35% sucrose, was placed on a sucrose barrier gradient (three 13-ml samples of 80, 65, and 35% sucrose layered, in that order, in a 50-ml centrifuge tube) which was centrifuged for 2 hr in swinging cups at 2,400 × *g*. The bacteria were predominantly in a layer whose base was the 80% sucrose level, and which occupied approximately one-third of the 65% sucrose level. This layer was found to be still contaminated with gross tissue debris. This could, however, be easily removed by filtration through a 10-μ membrane filter (Millipore Filter Corp., Bedford, Mass.) and subsequent high-speed centrifugation of the filtrate to concentrate the bacteria. This treatment resulted in a preparation relatively free from tissue components. Final recovery in terms of viable count was 6.4% of the number in initial homogenate. Since viable salmonellae per organ at death are 10^8 per liver to 10^9 per spleen (E. Schewe, J. Infect. Diseases **102**:275, 1958), such recovery barely suffices, unless large numbers of animals are used, to yield sufficient organisms for most physiological or composition studies of the bacteria. This study points up the problems involved in obtaining pure suspensions of such organisms essentially free from tissue debris, especially the multitudinous mitochondria, and presents a method utilizing a minimal number of manipulations within a reasonably short period of time.

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