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The Action of Sodium Deoxycholate on *Escherichia coli*

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Sodium deoxycholate is used in a number of bacteriological media for the isolation and classification of gram-negative bacteria from food and the environment. Initial experiments to study the effect of deoxycholate on the growth parameters of *Escherichia coli* showed an increase in the lag time constant and generation time and a decrease in the growth rate constant and total cell yield of this microorganism. Cell fractionation studies indicated that sodium deoxycholate at levels used in bacteriological media interferes with the incorporation of [U-^{14}C]glucose into the cold-trichloroacetic acid-soluble, ethanol-soluble, and trypsin-soluble cellular fractions of *E. coli*. Finally, sodium deoxycholate interfered with the flagellation and motility of *Proteus mirabilis* and *E. coli*. It would appear then that further improvement of the deoxycholate medium may be in order.

The recommended methods for monitoring clinical specimens or the environment for enteric bacilli call for the utilization of differential and selective culture media, some of which contain sodium deoxycholate as an inhibitory agent against gram-positive bacteria (5). Gram-negative enteric microorganisms have been shown to be relatively resistant to detergents, which do not seem to disrupt their outer membranes (8). Sodium deoxycholate is supposed to be innocuous to gram-negative enteric bacilli, but it may influence the sensitivity of these bacteria to antibiotics or other agents (2, 3, 5). Because detailed, quantitative studies concerning the action of deoxycholate on the growth rate of and entrance of nutrients into enteric bacilli are needed, an attempt has been made in this investigation to address this need.

The aim of this study was to conduct a quantitative assessment of the influence of sodium deoxycholate on certain germane physiological parameters of *Escherichia coli*, which is used as an indicator of fecal pollution of water supplies.

E. coli ATCC 25922 and a strain of *Proteus mirabilis* isolated from clinical material were obtained from the stock collection of the clinical microbiology laboratory at Loyola University Medical Center. Stock cultures were maintained on Trypticase soy agar (BBL Microbiology Systems) slants at 4°C and were transferred to new slants every 4 weeks. Cultures were tested periodically for gram reaction; indole production; citrate utilization; glucose, sucrose, and lactose fermentation; reaction on triple sugar iron medium; and growth on eosin-methylene blue and MacConkey agars. Antibiograms were determined by the Bauer-Kirby method (1).

An overnight culture grown in Trypticase soy broth at 37°C was harvested by centrifugation and washed in saline, and a homogenous suspension was made by manually shaking the culture for 15 min in an Erlenmeyer flask containing glass beads (3-mm diameter). The density was adjusted to 10 Klett units by the use of a Klett-Summerson photoelectric colorimeter with a no. 42 filter. From this stock solution an inoculum of 5×10^2 CFU/ml of *E. coli* was used to reflect the low levels of gram-negative bacilli that are usually encountered in water or food. The flasks were incubated at 37°C and titrated at intervals by the pour plate method with Trypticase soy agar as the plating medium. Colonies developing after 48 h of incubation at 37°C were counted. All samples were run in triplicate. Growth parameters were determined by the method of Monod (7).

An examination of growth parameters (Table 1) showed that the lag time constant, growth rate constant, and generation time were all significantly affected at least at the 5% level in *E. coli* (10). The growth rate constant represents the slope of the growth curve, that is, the change in the $\log_{10}$ cell population over the change in time (7). Similar data were obtained for *P. mirabilis*.

In an attempt to obtain additional data concerning the affect of deoxycholate on the growth of *E. coli*, we used the procedure of Park and Hancock (9) to study the action of deoxycholate on the entrance and incorporation of [U-^{14}C]glucose into five cellular fractions of *E. coli* (11). The cold-trichloroacetic acid (TCA)-soluble fraction contains the amino acids and other low-molecular-weight compounds found in the cellular pool; the ethanol-soluble fraction contains cellular lipid and very small amounts of alcohol-soluble protein. Hot TCA removes the nucleic acids. Trypsinization converts over 95% of the cell protein to soluble peptides. The residue fraction consists predominantly of the cell wall mucopeptides. An 18-h Trypticase soy broth culture was harvested, washed twice with saline, and suspended in 0.1 M sodium phosphate buffer (pH 7.0) at a density of 200 Klett units by use of a Klett-Summerson photoelectric colorimeter with a no. 42 filter. To 100 ml of bacterial suspension 1.35 μCi of D-[U-^{14}C]glucose (specific activity, 237 mCi/mmol) was added. After being labeled for 30 min, the cells were harvested, washed, and suspended in 0.1 M phosphate buffer (pH 7.0) at a density of 200 Klett units. The cells were distributed in four tubes, deoxycholate was added at final concentrations of 0, 0.05, 0.1, and 0.2% (wt/vol), and the mixture was incubated at 37°C for 24 h. The bacteria were then fractionated by the method of Park and Hancock (9), and the distribution of the radioactive label into the cellular fractions of *E. coli* was determined (11).

Table 2 shows that deoxycholate caused a leakage of radioactivity from unfractionated cells of *E. coli* that was proportional to the concentration of deoxycholate. When *E. coli* was fractionated (9), it was found that in the presence of 0.1% deoxycholate the incorporation of ^{14}C into the cold-TCA-soluble fraction was reduced from 22,500 to 8,350 cpm. The ^{14}C activity of the ethanol-soluble fraction was reduced from 16,850 to 9,750 cpm. The ^{14}C activities of the hot-TCA

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TABLE 1. Effect of sodium deoxycholate on the growth parameters of *E. coli* ATCC 25922[a]

Sodium deoxycholate concn	Lag time constant[b]	Growth rate constant[c]	Generation time (min)[d]
None (control)	119 ± 6.2	0.99 ± 0.02	17.5 ± 0.93
1,000 μg/ml	154 ± 16.4	0.73 ± 0.04	24.7 ± 1.14

[a] Values are means ± standard deviations.
[b] $P < 0.05$ (significant).
[c] $P < 0.001$ (significant).
[d] $P < 0.001$ (significant).

and trypsin-soluble fractions were reduced from 33,000 to 19,750 cpm and from 17,900 to 13,240 cpm, respectively. The incorporation of ^{14}C into mucopeptides was unaffected.

Leifson (5) states that *Proteus* colonies usually do not spread on media containing 0.1% deoxycholate. To assess precisely the effect of deoxycholate on flagellum formation, we used electron microscopy to examine cell suspensions of *P. mirabilis* or *E. coli* grown in the presence or absence of deoxycholate. Bacteria were grown on deoxycholate agar plates with or without the sodium deoxycholate component. After 18 h of incubation at 37°C, a suspension was made in distilled water, and a small drop of the suspension was applied to 200 mesh copper grids coated with 0.25% Formvar. The cells were stained with 5% (wt/vol) uranyl acetate for 10 min, and excess stain was removed by rinsing in distilled water. Micrographs were taken with an RCA EM U 3 electron microscope at 50 kV (4). The results of these experiments are shown in Fig. 1a and b. A reduction in the number of flagella per bacterial cell was observed when the assay microorganisms were grown in the presence of 0.05% (wt/vol) deoxycholate, and complete inhibition of flagellum formation was observed when the cells were grown in the presence of 0.1% deoxycholate. Furthermore, when *P. mirabilis* was inoculated on deoxycholate agar plates containing 0.05 or 0.1% deoxycholate, complete inhibition of swarming was observed even after 32 h of incubation at 37°C. On the control plates containing the deoxycholate basal medium (without deoxycholate), swarming was apparent after 6 to 8 h, whereas on the plates containing 0.025% deoxycholate, swarming was apparent after 14 h (Fig. 2a to d).

The results presented in this paper indicate that sodium deoxycholate at the concentrations used in bacteriological media interferes with the growth and incorporation of glucose into various cellular fractions of *E. coli*. Substances taken into cells and incorporated into cellular components must pass through metabolic pools before entering the various metabolic pathways (9). In this way, pool sizes may directly influence the synthesis of macromolecules.

The inhibition of flagellum formation by 0.1% deoxycholate in the assay microorganisms may be responsible for the decreased spreading of these bacteria. It is possible that the flagellum protein (flagellin), the hooks, and the basal structures were being synthesized but could not be assembled in the presence of deoxycholate. Another possibility is that the apparatus for flagellum synthesis is separate from that responsible for the synthesis of cellular proteins. This possibility is in agreement with the findings of McClatchy and

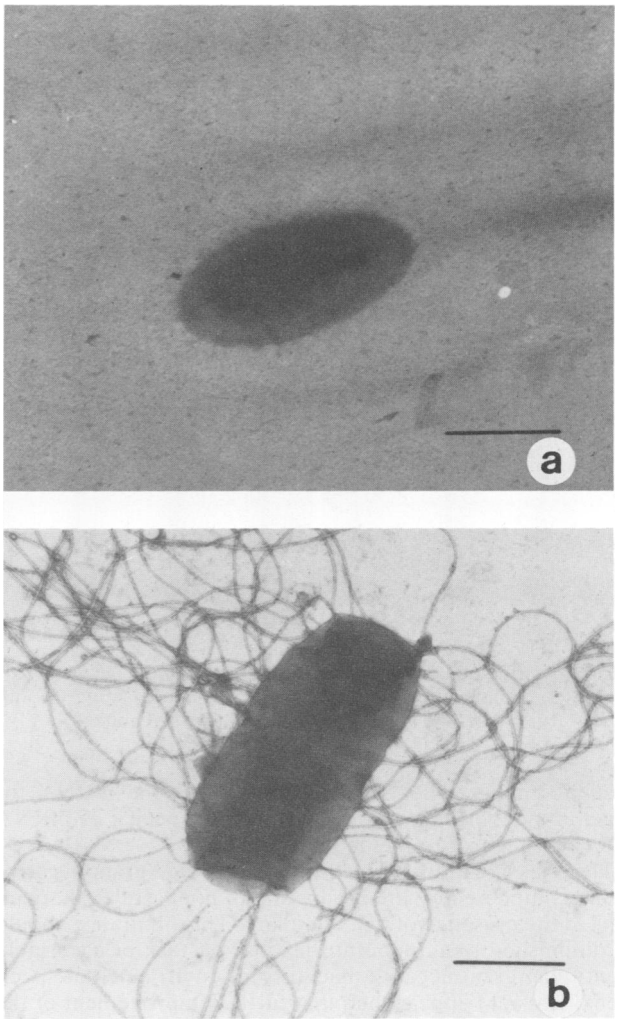

FIG. 1. (a) Electron micrograph of *P. mirabilis* grown on deoxycholate agar containing 0.05% sodium deoxycholate. Bar, 1 μm. (b) Electron micrograph of *P. mirabilis* grown on deoxycholate basal medium (control). Bar, 1 μm.

TABLE 2. Effect of deoxycholate on labeled cellular fractions of *E. coli*

Deoxycholate concn (% [wt/vol])	cpm/total volume of indicated fraction:					
	Cold-TCA soluble	Ethanol soluble	Hot-TCA soluble	Trypsin soluble	Residue	None (unfractionated cells)
0.00	22,500	16,850	33,000	17,900	36,050	139,650
0.05	10,950[a]	11,300[a]	26,500	14,720	36,150	116,250
0.10	8,350[a]	9,750[a]	19,750[a]	13,240	36,240	114,000[a]
0.20	5,350[a]	5,600[a]	14,000[a]	12,280	32,400	75,000[a]

[a] $P < 0.01$.

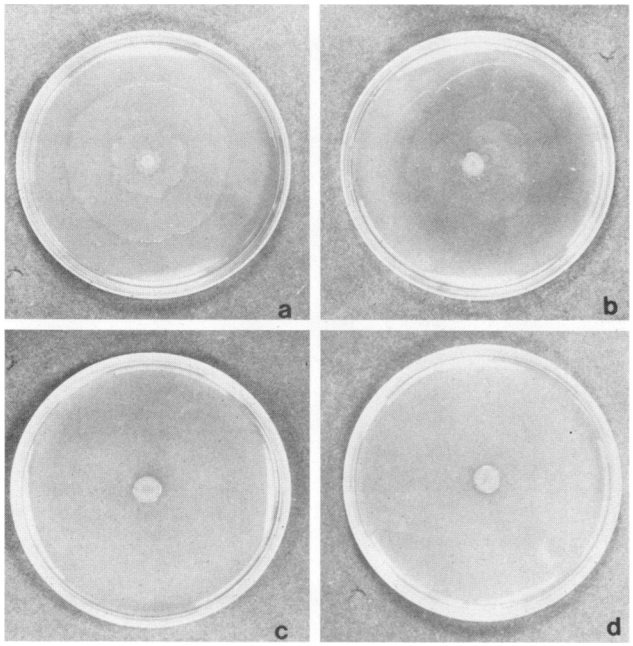

FIG. 2. Effect of deoxycholate on the swarming of *P. mirabilis* after 24 h of incubation on deoxycholate agar containing 0% (a), 0.025% (b), 0.05% (c), and 0.1% (d) sodium deoxycholate.

Rickenberg (6), who have suggested that the mRNA for flagellin synthesis is stable and that the species of mRNA vary with respect to metabolic stability.

The classification of well-known motile, gram-negative bacilli is hindered by the production of nonflagellated organisms in the presence of deoxycholate. This hindrance should be diminished to aid laboratories in their task of accurately identifying gram-negative bacilli from environmental specimens. It would appear then that further improvement of the deoxycholate medium which is used for the isolation of gram-negative enteric bacilli from food and water may be in order. This improvement may be achieved by a search for a detergent which will be inocuous to the growth or flagellation of the assay microorganisms.

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