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Inhibition of Hemolytic Activity of El Tor *Vibrio* by Antibiotics Which Interfere with Protein Synthesis* (32644)

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The inhibition of protein synthesis by antibiotics such as tetracycline, chloramphenicol or erythromycin has been found to be quite selective. Chloramphenicol and erythromycin, for example, suppress protein synthesis and the adaptive formation of beta-galactosidase; inhibition of the induction of this enzyme in *Escherichia coli* occurs at concentrations of antibiotic that permit exponential growth (1, 2). Oxytetracycline has been noted to interfere preferentially with the induction of penicillinase in *Staphylococcus aureus* (3).

The purpose of the study reported in this paper was to investigate the possibility of selective inhibition of the hemolytic activity of the El Tor strain of *Vibrio cholera* by antibiotics that alter protein synthesis. The data obtained indicate that, under certain conditions, inhibition of hemolysis may follow exposure to some antimicrobial agents without the development of obvious changes in the growth characteristics of the organism.

Materials and Methods. The El Tor strain of *V. cholera* used in these studies was obtained from American Type Culture Collection and was maintained in the laboratory by repeated passages on 5% horse blood agar.

The antibiotics employed included conventional commercial preparations of penicillin, streptomycin, polymyxin, tetracycline and oleandomycin, paromomycin, chloramphenicol, erythromycin, cephalothin, kanamycin and colistin. Varying concentrations of each compound were prepared in heart infusion broth. These were mixed with a 10^{-3} dilution of an 18-hour broth culture of *V. cholera* in equal volume and incubated at 37°C for different periods of time. Deep and surface subcultures from these mixtures were made on 5% horse blood agar and incubated at 37°C overnight, at which time the presence or absence of beta-hemolysis was noted.

Results. Inhibition of hemolysis by single antibiotics. Subcultures of *V. cholera* from broth containing tetracycline, chloramphenicol, oleandomycin, erythromycin and paromomycin failed to produce hemolysis. The degree of inhibition of hemolytic activity appeared to be related to the concentration of the drug and the length of time over which the organism was exposed to it. As shown in Table I, inhibition of hemolysis was demonstrable only when the level of the antibiotics exceeded their minimal inhibitory concentration (MIC). Tetracycline and chloramphenicol were effective at 5–10 times their MIC, whereas the other antimicrobiol agents were active only at 10 times their MIC.

That the duration of exposure of the organism to a drug was of importance in suppressing its hemolytic activity is illustrated by the data presented in Table II. Production of

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TABLE I. Relation of Concentration of Antibiotics to Inhibition of Hemolysis.

Antibiotic	Hemolysis—concentration of drug			
	10 MIC[a]	5 MIC	2 MIC	< MIC
Tetracycline	0	0	+	+
Chloramphenicol	0	0	±	+
Paromomycin	0	+	+	+
Oleandomycin	0	+	+	+
Erythromycin	0	+	+	+

[a] Number of times the minimal inhibitory concentration; + = hemolysis; ± = partial hemolysis; and 0 = no hemolysis.

the hemolysin appeared to be inhibited during a short period when the antibiotics were exerting antibacterial effects most actively. Inhibition of hemolysis by tetracycline and chloramphenicol developed just before the number of bacteria began to decrease. Prior to this, hemolysis was partially suppressed; this became complete as the size of the bacterial population fell. Examination of the texture and morphology of hemolytic and nonhemolytic colonies revealed no differences. Although enumeration of the organisms growing in broth containing paromomycin, oleandomycin, or erythromycin was not carried out, inhibition of hemolysis appeared to begin at a time just before a decline was apparent in the numbers of colonies appearing on subculture on agar. As with the other drugs, suppression of hemolytic activity seemed to be associated with a significant decrease in the number of viable bacteria.

Kanamycin, polymyxin, and colistin produced no effects on hemolysin production. Exposure to penicillin and cephalothin appeared to increase the hemolytic activity of *V. cholera*.

In order to rule out the possibility that the antibiotics were merely selecting out nonhemolytic mutants, the colonies exhibiting absence of hemolysis were immediately subcultured in drug-free media. All were actively hemolytic on the first transfer. This suggests that the nonhemolytic colonies were exhibiting only a temporary change, rather than a stable or genetic alteration.

Effect of combinations of antibiotics on hemolysis. The addition of penicillin or cephalothin to tetracycline or chloramphenicol did not prevent the inhibition of hemolysis by the latter but altered it to some degree. Mixtures of cephalothin with either of the broad-spectrum antibiotics shortened the time required for suppression of hemolytic activity from 4 to 6 hours (tetracycline or chloramphenicol alone) to 2 hours. The presence of penicillin decreased the degree of inhibition of hemolysin production. Enumeration of bacteria by the plate-counting method 2, 4, and 6 hours after exposure to cephalothin alone or to combinations of this compound with tetracycline or chloramphenicol, revealed that the degree of reduction in the numbers of organisms was not significantly different. The effects of the drug mixtures on hemolysin production were also not different from those of the single agents, except for acceleration

TABLE II. Inhibition of Hemolysis in Relation to Duration of Exposure to Antibotics (10 MIC).

Antibiotic	Exposure to antibiotic (hours)					
	2		4		6	
	Hemolysis[a]	Bacterial count	Hemolysis	Bacterial count	Hemolysis	Bacterial count
Tetracycline	±	10^1	0	5×10^2	0	11
Chloramphenicol	+	4×10^1	±	3×10^1	0	2×10^2
Paromomycin	0	—	+	—	+	—
Oleandomycin	0	—	0	—	+	—
Erythromycin	+	—	+	—	0	—
No drug	+	58×10^5	+	4×10^8	+	3×10^9

[a] + = Complete hemolysis; ± = Partial hemolysis; and 0 = No hemolysis.

TABLE III. Effect of Antibiotic Combinations on Hemolysis.

Antibiotic[a]	Exposure to antibiotic (hours) 2 Hemolysis[b]	2 Bacterial count	4 Hemolysis	4 Bacterial count	6 Hemolysis	6 Bacterial count
PN	+	2×10^5	+	2×10^4	+	10^4
CP	+	1.6×10^3	+	8×10^3	+	2×10^3
CM	+	4×10^4	±	3×10^4	0	2×10^3
TC	±	1.5×10^4	0	50	0	11
PN + CM	+	7×10^4	±	2×10^4	±	3×10^4
PN + TC	±	2×10^4	0	40	±	8
CP + CM	0	9×10^4	±	1.2×10^6	+	3×10^4
CP + TC	0	2×10^4	0	30	±	6
No drugs	+	3×10^6	+	2×10^8	+	3×10^9

[a] 10 MIC of antibiotic, either alone or in combination; PN = penicillin G; CP = cephalothin; CM = chloramphenicol; and TC = tetracycline.

[b] 0, ±, + = No, partial, or complete hemolysis.

of the development of hemolysin inhibition.

Discussion. The El Tor strain of *V. cholera* produces a lecithinase and another hemolysin (4,5). While the lecithinase is a protein, the other hemolytic factor has been found to consist of 95% lipid and 5% protein; both of these appear to be necessary for hemolytic activity (6). The results of the present studies indicated that exposure of this organism to antibiotics that block protein synthesis inhibited its capacity to produce hemolysis. Antimicrobial agents that affect cell wall synthesis or cell membrane integrity appeared to be without effect. The hemolytic-suppressive activity was related to the concentration of the drug and the time over which the organism was allowed to remain in contact with it. That inhibition of hemolysin may be the result of a highly selective effect of an antibiotic is suggested by the fact that it was noted to appear before and to develop fully at a time when the numbers of bacteria had just begun to decrease, and that the colonial morphology of hemolytic and nonhemolytic populations were indistinguishable.

Penicillin and cephalothin appeared to have the capacity to potentiate the hemolytic activity of the El Tor strain of *V. cholera*. An enhancement of the production of staphylococcal alpha hemolysin by penicillin has also been reported (7). The addition of penicillin to either tetracycline or chloramphenicol did not alter the time when inhibition of hemolysis occurred, but decreased the degree of the effect; this suggests the possibility of neutralization of two opposed activities. These results are contrary to those suggesting that a decrease in the antibacterial activity of penicillin follows the addition of tetracycline or chloramphenicol to it (8). However, they agree with data indicating that a decrease in cytotoxicity may be observed when either of the broad-spectrum agents is mixed with penicillin (9). The differences in the effects of cephalothin and penicillin on hemolysin production by *V. cholera* are difficult to explain in view of the similarities of these drugs in terms of their mechanism of action, antibacterial spectrum, and interaction with broad-spectrum antimicrobial compounds (8–11).

Summary. Exposure of the El Tor strain of *V. cholera* to antibiotics which interfere with protein synthesis was found to suppress the capacity of this organism to produce hemolysis. Inhibition of this effect appeared just prior to and in the early phase of decrease in the numbers of bacteria. Inhibition of hemolysin production was related to the concentration of antibiotic and the time over which the organism remained in contact with it. Antimicrobial agents which act by interfering with cell wall synthesis or alter the integrity of cell membranes did not decrease hemolysin production; penicillin and cephalo-

thin appeared to increase it. The addition of penicillin to tetracycline or chloramphenicol reduced the hemolytic inhibitory activity of these compounds. Combining cephalothin with these broad-spectrum antibiotics shortened the time required for inhibition of hemolysis to develop.

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The 1- and 2-Octadecyl Glyceryl Ethers as Model Compounds for Study of Triglyceride Resynthesis in Cell Fractions of Intestinal Mucosa* (32645)

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It has been well documented that dietary triglycerides are split in the lumen of the intestine into 2-monoglycerides and free fatty acids. Further, it has been shown that the monoglyceride penetrates the intestinal mucosa and in the mucosal cell is resynthesized to triglyceride (1). In the hamster intestine, according to Johnston (2), both the 1- and 2-monoglycerides are readily converted into triglyceride via the 1,3- and 1,2-diglyceride intermediates, respectively. In the rat intestine, the 2-monoglyceride has been reported to be the preferred acyl acceptor in the formation of triglyceride (3,4), but such data is difficult to evaluate since the 2-monoglyceride rapidly undergoes isomerization to the 1-monoglyceride. Likewise, the ease with which the 1,2-diglyceride isomerizes to the 1,3-diglyceride has made determination of the intermediate diglyceride difficult.

Swell *et al.* (5) have shown that both the 1- and 2-octadecyl glyceryl ethers are absorbed by rat intestine *in vivo* and appear in the lymph as alkoxydiglycerides. Sherr and Treadwell (6), using everted rat intestinal sacs, have demonstrated the conversion of 2-octadecyl glyceryl ether into alkoxymono- and alkoxydiglycerides. Kern and Börgstrom (7), using hamster intestinal rings, have shown that both the 1- and 2-monoglyceryl ethers are converted into alkoxydiglycerides as readily as monoolein is converted into triglyceride. The purpose of the work presented here was to establish (a) which monoglyceride isomer is preferred for triglyceride resynthesis, and (b) the positional specificity of the diglyceride intermediate for its acylation to triglyceride using octadecyl glyceryl ethers as model compounds. The glyceryl ethers are useful since they fail to undergo isomerization or significant splitting in the *in vitro* system but are converted to alkoxydiglycerides apparently by the same mucosal transacylases which convert monoglyceride into triglyceride (7).

Experimental. Male Wistar rats from Carworth Farms, weighing between 100 and

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