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# *Vibrio cholerae* Hemagglutinin/Protease Nicks Cholera Enterotoxin

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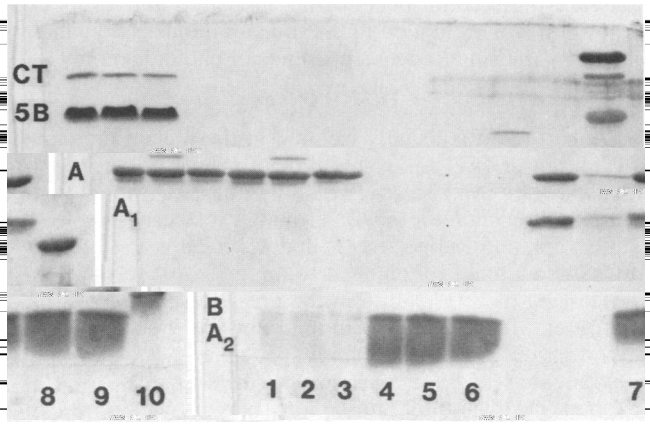
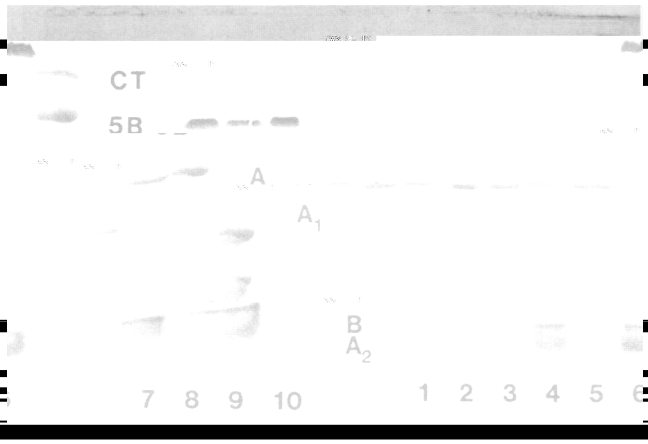
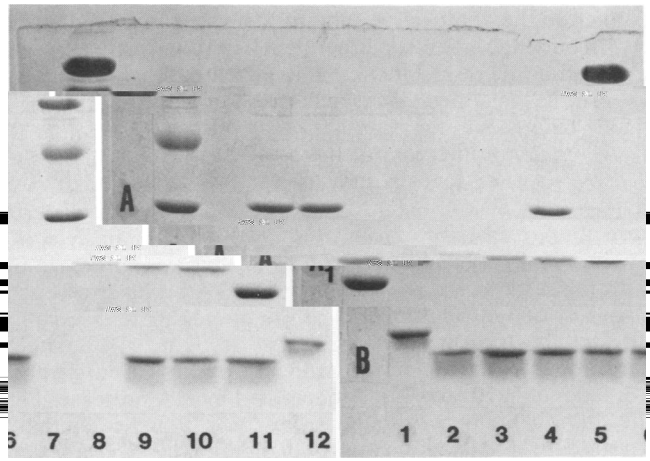
**Unnicked cholera enterotoxin was isolated from culture supernatants of *Vibrio cholerae* 569B by either rapid processing of flask-grown cultures or by growing and processing fermentor cultures in the presence of ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetra acetic acid, an inhibitor of the previously described *V. cholerae* hemagglutinin/protease. When unnicked cholera enterotoxin was incubated with purified hemagglutinin/pro-**

$10^{10}$/ml. Partially purified enterotoxin was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis with




12% polyacrylamide gels. A significant proportion of the A subunit was unnicked when flask-grown cultures with or without EGTA (or 150 μg of Zincov per ml [MIC, 3 mg/ml]; data not shown) were rapidly processed, i.e., within about 6 h (Fig. 1, lanes 8 and 9). When toxin was produced in fermentor-grown cultures that were then processed at room temperature during the next 2 days, the enterotoxin produced and processed in the absence of EGTA was almost




the culture grown in the presence of 6.6 mM EGTA and processed in the presence of 1 mM EGTA was almost completely unnicked (Fig. 2). Thus, the chelating agent protected the cholera toxin from being nicked. However, when this unnicked, partially purified cholera toxin was left

centration was estimated by a crude immunoassay, which is biased by the simultaneous presence of choleragenoid (3, 7).

## DISCUSSION

Cholera toxin is usually isolated in the nicked (activated) form. Addition of EGTA, an inhibitor of HA/protease, can

peptide bonds upstream (12) from the cleavage site is an Arg-Ser bond, a likely site of trypsin hydrolysis that would yield a slightly smaller $A_1$ fragment. It is also possible that other proteases may effect activation by nicking neighboring peptide bonds.

In summary, although gut enzymes may play a contribu-