Diphtheria toxin undergoes membrane insertion and translocation across membranes when exposed to low pH. In this study, the translocation of the toxin has been investigated by the binding of antibodies to two preparations of model membrane-inserted toxin. In one preparation, toxin was added externally to model membrane vesicles and then inserted by exposure to low pH. In the other preparation, toxin was entrapped in the vesicles at neutral pH, and then inserted by decreasing pH. At neutral pH, externally added antibodies could not bind to entrapped toxin, although they could bind to externally added native toxin. However, after low pH exposure, antibodies against all three toxin domains (catalytic (C), transmembrane (T), and receptor-binding (R)) could bind to entrapped toxin, and also to externally added membrane-inserted toxin. The binding to the entrapped toxin shows that all three domains of the toxin translocate to the trans face of the membrane after exposure to low pH. The observation that antibodies bind to both external and entrapped preparations of toxin after low pH exposure shows that toxin inserts in a mixed orientation.

A difference in antibody binding to low pH-treated toxin in which the C domain is folded (Lr conformation) or unfolded (Lr conformation) was also observed. An increase in antibody binding to C and T domains in the Lr conformation relative to binding to the Lr conformation was found for entrapped toxin, suggesting that more of the C and T domains translocate across the bilayer in the Lr conformation. These results suggest all three toxin domains insert in the membrane bilayer and participate in translocation in vitro. The C and R domains lack classical transmembrane hydrophobic sequences. However, they possess sequences that have the potential to form membrane-inserting β-sheets.

Diphtheria toxin contains 535 residues (M, 58,348) and comprises an A chain, which is equivalent to the catalytic (C) domain, and a B chain, which comprises transmembrane (T) and receptor-binding (R) domains (1). After endocytosis, the acid environment of the endosome lumen triggers a conformational change which allows the toxin to insert and then translocate the C domain across the membrane bilayer (2). The C domain is then released into the cytoplasm. Hydrophobic helices in the T domain are the most obvious sequences that may be involved in translocation (1). However, several studies have indicated that the C domain may also participate actively in membrane interaction and translocation (3–9). Furthermore, the R domain, which has been thought to act simply to bind to the receptor for the toxin, becomes hydrophobic at low pH (10), raising the possibility it has an important role in translocation.

In order to clarify the role each domain plays in translocation, monoclonal antibodies to the C, T, and R domains and polyclonal anti-peptide antibodies were used to examine the interaction of all three toxin domains with the membrane bilayer. A system was used in which the toxin is added externally to vesicles or entrapped within the vesicle lumen. This latter system is analogous to endosomes, allowing the detection of translocation (5, 11). The results show that all three domains traverse the bilayer and become exposed to the trans side of the membrane after exposure to low pH. This is a strong argument for the proposal that all three domains participate directly in translocation of the C domain in vitro. The observations that the unfolding of the C domain further promotes translocation and that the toxin inserts in a mixture of orientations are additional clues to the translocation mechanism.

EXPERIMENTAL PROCEDURES

Materials— Immunoplate MaxiSorp, flat-bottom 96-well microtiter plates were purchased from Nunc. Goat-anti rabbit IgG labeled with alkaline phosphatase was obtained by Southern Biotechnology Associates (Birmingham, AL). Goat-anti mouse IgG labeled with alkaline phosphatase was obtained by Jackson Laboratories (West Grove, PA) and Fisher BioTech. Sigma 104 phosphatase substrate (5 mg/ml tablets) and octyl glucoside (OG) were purchased from Sigma. DOPC and DOPG were obtained from Avanti Polar Lipids (Pelham, AL). Anti fluorescein whole antibody and F(ab’)2 fragments (1–4 mg/ml in 0.1 mM potassium phosphate, pH 8, with 2 mM sodium azide), rhodamine-DHPE, biotin-DHPE, biotin-X-DHPE, and streptavidin were obtained from Molecular Probes (Eugene, OR). Phast SDS-polyacrylamide gel electrophoresis gels and PhastSystem supplies were obtained from Pharmacia Biotech Inc. Nitrocellulose membranes were purchased from Schleicher & Schuell. Toxin was prepared as described previously (4, 13). Antibodies were prepared as described in accompanying paper except that they were diluted with Tris buffer (15 mM Tris-Cl, 150 mM NaCl, pH 7.2) prior to use. Antibody concentrations were identical to the antibody concentration reported in the accompanying article (12), except where noted.

Lipid Preparation and Entrapping Toxin—LUV with and without entrapped toxin were prepared by detergent dialysis as described previously.
LUV contained (w/w) 79.5% DOPC, 20.2% DOPG, and (as determined spectrophotocally at 563 nm using a ε = 73,000 m⁻¹ cm⁻¹) 0.1–0.3% of rhodamine-DHPE. A small aliquot was removed from each final preparation, and its rhodamine fluorescence (found to be insensitive to detergent) relative to the initial (pre-dialysis) mixture was measured to calculate lipid concentration. Toxin concentration was measured by competition ELISA with native toxin added externally to empty LUV used to prepare a standard curve (see below). To detect entrapped toxin by ELISA, acetyl glusclde (final concentration, 15 mg/ml) was added to the entrapped (and standard) samples to dissolve the vesicles. The percent of initially added toxin entrapped within the LUV was found to range between 5 and 10%. The toxin concentration ranged from 7.4 to 21.0 μg/ml (average 12.3 μg/ml) and the lipid concentration ranged from 1.10 to 2.74 mg/ml (average 1.72 mg/ml). Given the diameter of the vesicles (about 2500 Å; Ref. 5) and the toxin concentration used, about 65 toxin molecules are entrapped within each vesicle.

Demonstrating Entrapped Toxin in the Native Conformation Does Not React with Externally Added Antibodies—A competition ELISA protocol was used as in the accompanying article (12), with the exception that free dimer toxin was used as coating antigen. For α-T1 (see accompanying article for antibody nomenclature), 115 μl of toxin-containing vesicles were diluted to 200 μl with Tris buffer and then different aliquots were diluted with vesicles without toxin to maintain 1 mg/ml lipid concentration. For α-C2 and α-R1, 88 μl of toxin-containing vesicles were diluted to 10 μl with Tris buffer and then different aliquots were diluted with vesicles without toxin to maintain 1.7 mg/ml lipid concentration. Tris-diluted antibodies were added and samples incubated for about 2 h at room temperature. Samples were then diluted to 320 μl with Tris buffer, and 100 μl aliquoted into the ELISA plate as described previously (12). Final antibody concentrations were 0.20 μg/ml for α-T1, 0.98 μg/ml for α-C2, and 0.56 μg/ml for α-R1.

Demonstrating Entrapped Toxin Does React with Externally Added Antibodies after the Addition of Detergent—For experiments with α-T1, 9.4 μl of 240 mg/ml OG was added to a 115 μl aliquot of LUV-entrapped toxin or toxin added externally to LUV having the same protein and lipid concentrations to give an OG concentration of 15 mg/ml (for α-C2 and α-R1, 6 μl of OG was added to 88-μl toxin-LUV aliquots). For α-T1, 35 μl OG was added and the samples incubated at room temperature (this step was skipped for the other antibodies). Then for α-T1, 40 μl of additional Tris buffer was added (15 μl for α-C2 and α-R1) and incubated for 15 min. Tris-diluted antibodies were added and samples incubated for about 2 h at room temperature. Samples were then diluted to 320 μl with Tris buffer, and 100 μl aliquoted into the ELISA plate as described previously (12). Final antibody concentrations were as in the preceding section.

Assay for Antibody Permeation into the Lumen of Model Membrane Vesicles—66-kDa FITC-dextran (pre-dialyzed in 8-κDa dialysis tubing to remove small dextrans) was entrapped in LUVs with or without adenine-3-phosphate 5′-uridine phosphate-bound monomer (79.5% DOPC, 20.2% DOPG, and 0.12% pyrene-DHPE) as described above. Aliquots of 110 μl of 66-kDa FITC-dextran containing vesicles were incubated externally to LUV with added 200 mM sodium acetate, pH 4.4, and a FITC-dextran concentration of 0.39 μg/ml. The toxin concentration was measured spectrophotocally using ε580nm = 40,542 m⁻¹ cm⁻¹ was added and samples incubated 30 min at room temperature (13). 120 μl of each sample was centrifuged using a Beckman airfuge at 26 p.s.i. (95,000 rpm, 120,000 × g) for 30 min using an A-100 rotor. 100 μl was removed and washed three times, using 100 μl of Tris buffer followed by a 20-min spin for each wash. After the final spin, 100 μl was removed and 20 μl of Tris buffer and 10 μl of 110 mg/ml OG was added. After the pellet was completely dissolved by vortexing, 15 μl of the sample was added to a 5-μl solution of 50 μl Tris-CI, 1 μl Na2EDTA, 2.5% (w/v) SDS, 0.01% (w/v) bromphenol blue, pH 8.0, and 7.5% (w/v) 2-mercaptoethanol) and boiled for 10 min. Then a 2-μl aliquot of each sample was run on a 4–15% PhadGel using the PhastSystem apparatus, transferred to a nitrocellulose membrane and detected as in the accompanying paper (12).

RESULTS

Preparations Used to Examine the Topography of Membrane-Entrapped Toxin—Antibody binding was used to evaluate the conservation of diphtheria toxin inserted in model membranes. Fig. 1 schematically illustrates the preparation of the two types of model membrane vesicles containing toxin that were examined. In one preparation, the toxin was added externally to (outside of) model membrane vesicles, and then inserted by exposure to low pH. In the other preparation, the toxin was
Antibodies Do Not Bind to Entrapped Native Toxin—Competition ELISA. Entrapment of toxin was prepared by detergent dialysis. Membrane-inserted toxin was prepared by the low pH treatment of toxin added externally to LUV and toxin entrapped within the aqueous lumen of an LUV. The side of membrane insertion is considered the cis side, and the opposite side of membrane insertion is considered the trans side. Notice that a given site (illustrated as a black dot at the apex of toxin molecule) is exposed on the external surface in only one preparation when orientation is unidirectional.

![Diagram of antibody binding to membrane-inserted toxin](image)

In these experiments fluorescein conjugated to a 66-kDa dextran was entrapped with or without toxin in the lumen of model membrane vesicles. After exposure to low pH to insert toxin, and the addition of anti-toxin antibodies where desired, the α-FITC was added and the fluorescein fluorescence was monitored. Table I shows samples with entrapped toxin and FITC-dextran do not form pores large enough to release 66-kDa FITC-dextran or allow α-FITC to enter into the vesicle lumen even after exposure to low pH. This was true for toxin in both the Lr° (23°C) conformation, in which the C domain is folded, and Lr° (37°C) conformation, in which the C domain is partially unfolded.

The relative strength of binding for different antibodies is summarized in Table II. One observation is that after membrane-inserted toxin was prepared by the low pH treatment of either toxin added externally to vesicles or entrapped toxin. Experiments were performed both with low pH-treated toxin in the Lr° (23°C) conformation, in which the C domain is folded, and Lr° (37°C) conformation, in which the C domain is partially unfolded (4).

2 The possible weak binding seen in the entrapped preparation might be due to a small amount of toxin that had leaked out of the vesicles. Extrapolation of the inhibition curves to determine the amount of toxin that inhibits ELISA by 50% (IC50) for entrapped toxin and comparison to the IC50 after detergent release can be used to estimate the amount of toxin outside of the vesicles. This gives an upper limit of 5–10% of the toxin in the entrapped preparation, after correction for the effect of OG on antibody binding. (The correction varied from no effect for α-R1 to a 2-fold difference for α-T1.) This percent is much too small to account for the amount of reaction seen with the entrapped toxin after low pH treatment.

3 Similar experiments were performed with an α-FITC Fab, showing that after low pH-induced toxin insertion and pH neutralization, the Fab, which are slightly smaller than toxin (about 50 kDa), was also unable to permeate the vesicles (data not shown).

4 We have changed our previously assigned names for the conformations the toxin takes after low pH treatment and pH neutralization from R° and R° to Lr° and Lr°, respectively. This was done to avoid confusion between the R domain and R conformation.
A 90% decrease of fluorescein fluorescence upon addition of toxin is observed, there must be a decrease in intensity of fluorescein dextran fluorescence by 10% as judged by a release of fluorescein from vesicles following exposure to low pH, as judged by a 10% decrease in intensity of fluorescein dextran fluorescence. This was ruled out by experiments in which both toxin and FITC-dextran were entrapped in vesicles and then antibody binding to the trans face of the membrane. The IC$_{50}$ values obtained are generally comparable to those observed in other experiments (5). No additional intensity decrease was observed after pH neutralization. The release at low pH probably reflects a somewhat larger pore size as compared to after pH neutralization.

**TABLE I**

| Vesicle sample | Relative fluorescence of trapped 66-kDa FITC-dextran (b,c,d) | 5 min | 60 min | 120 min | (+10G) |
|---------------|-------------------------------------------------|-------|--------|---------|--------|
| Entrained toxin without $\alpha$-toxin           | 99 (99)                                       | 98 (95) | 10 (10) |
| Entrained toxin with $\alpha$-C1                  | 98 (100)                                      | 98 (101) | 98 (103) | 13 (8) |
| Entrained toxin with $\alpha$-T2                   | 99 (96)                                      | 99 (100) | 99 (101) | 9 (8)  |
| Entrained toxin with $\alpha$-R1                   | 99 (98)                                      | 98 (98) | 97 (99) | 9 (10) |
| Externally added toxin with $\alpha$-T2            | 97 (102)                                      | 97 (99) | 97 (105) | 13 (12) |

The concentrations of antibodies and membrane-inserted toxin used in these experiments are generally similar implies that there is a large fraction of membrane-inserted toxin molecules facing different directions or a mixture of membrane-inserted toxin molecules facing the cis and trans sides.

Another observation evident from the antibody binding data is that all of the anti-toxin antibodies are able to bind to both membrane-inserted toxin and the externally added and entrapped toxin preparations. The only way this could occur is if the toxin inserts into a mixture of orientations, such that some toxin molecules have sites facing the opposite side of the membrane, and others in the same sample have the same sites facing the opposite side of the membrane (see schematic figures). The observation that the IC$_{50}$ for antibody binding to entrapped and externally added toxin preparations are generally similar implies that there is a large fraction of toxin molecules facing each orientation. This result could be interpreted in terms of a mixture of transmembrane-toxin molecules facing in different directions or a mixture of transmembrane-toxin and toxin that only penetrates the membrane incompletely.

The IC$_{50}$ is on the average somewhat lower for externally added toxin than for entrapped toxin in the Lr$^+$ conformation, and averaging the difference in IC$_{50}$ values for externally added and entrapped toxin suggests that about 70% of the molecules have sites oriented facing the cis side of the membrane and 30% are oriented facing the trans side in this conformation. However, the difference in IC$_{50}$ could potentially be influenced by a difference in reactivity as well as the number of sites facing cis and trans sides.

**Antibody Binding to Membrane-inserted Toxin: Effect of Toxin Conformation**

- **Monoclonal antibody binding to membrane insertion of toxin by exposure to low pH, antibodies can bind to the entrapped toxin preparation. Since toxin remains membrane-bound (5), this reflects the exposure of sites in membrane-inserted toxin to the trans face of the membrane. The IC$_{50}$ values obtained are generally similar to those obtained for toxin in solution (see accompanying article (12)), indicating that the translocation of a large fraction of toxin molecules occurs. Furthermore, since interaction of $\alpha$-C, $\alpha$-T, and $\alpha$-R antibodies with the toxin is observed, there must be translocation of all three domains to the trans surface of the vesicle.**

- **To see if the toxin concentration affects this translocation, toxin was entrapped at lower toxin-to-vesicle ratios than in the protocol used above (65 toxin molecules/vesicle), and the binding of antibodies $\alpha$-C2, $\alpha$-T2, and $\alpha$-R2 was measured. After low pH treatment of toxin entrapped at 6.5, 3.2, or 1.6 toxin molecules/vesicle, binding of all three antibodies was observed (data not shown).**

5 An unlikely alternate interpretation of both the FITC-dextran experiments described earlier and the observation that entrapped toxin binds antibodies is that FITC-dextran binding to toxin blocks pore formation, and binding of antibody to entrapped toxin is due to pore formation and penetration of antibodies to the lumen of the vesicles. This was ruled out by experiments in which both toxin and FITC-dextran were entrapped in vesicles and then antibody binding to the entrapped toxin was measured. It was found that FITC-dextran did not significantly alter IC$_{50}$, whereas a large increase in IC$_{50}$ would be expected if FITC-dextran was preventing antibody entry into the vesicles (data not shown).
branefield inserted toxin in the Lr" and Lr" conformations was examined. In general, binding is slightly tighter when the toxin is in the Lr" conformation, as judged by a lower IC50. α-C2 behavior is somewhat different (see Table I), showing closer to equal binding to Lr" and Lr" binding. Binding of the anti-peptide antibodies α-C141-157 and α-T224-237 to toxin differs from that of the monoclonals in that binding to the Lr" toxin is tighter than binding to the Lr" toxin. It is possible that the increased degree of unfolding in the Lr" conformation promotes the binding of anti-peptide antibodies by increasing the exposure of peptide epitopes, whereas the non-contiguous surface epitopes seen for monoclonal antibodies (17, 18), are somewhat disrupted by the increased unfolding in the Lr" toxin.

Antibody Binding to Membrane-inserted Toxin: Evidence That Increased Translocation of C and T, but Not R Domain, Occurs upon C Chain Unfolding—The IC50 values in Table I show that for externally added toxin most antibodies bind the Lr" conformation toxin more tightly than the Lr" conformation toxin. The weaker binding of an antibody to its epitope on externally added toxin in the Lr" conformation may be explained in one of two ways: 1) there is a structural change of an epitope to a weaker binding form in the Lr" conformation, or 2) in the Lr" conformation the epitope was translocated to the trans side of the membrane bilayer (and so faces the antibody inaccessible vesicle lumen for most of the toxin molecules). It is possible to distinguish these possibilities by comparing antibody binding to entrapped toxin in the Lr" and Lr" conformations. If the decrease in antibody binding to toxin in Lr" conformation were due to a change in epitope structure, antibody binding would show a decrease in binding to Lr" toxin similar to that seen with externally added toxin. However, if the weaker reactivity of the Lr" toxin is due to translocation of the epitope across the bilayer, the ratio of reactivity of the entrapped Lr" toxin relative to Lr" toxin should be higher than for externally added toxin.

The second explanation is seen to be correct for several epitopes when IC50 values are examined. Table III shows the IC50/Lr"/IC50/Lr" ratio is lower for entrapped toxin than for externally added toxin in several cases, i.e. the reactivity of several antibodies with Lr" toxin normalized to the reactivity of the Lr" toxin is higher in the entrapped preparation than in the externally added preparation. We have defined the change in the Lr"/Lr"IC50 ratio as the relative translocation index. This is a measure of how much a site moves to the trans face upon unfolding of the C domain, the major change in the toxin conformation on going from Lr" to Lr" conformation (5). Interestingly, a translocation index close to 2, indicating increased translocation for toxin in the Lr" conformation, is seen for the anti-C and some anti-T antibodies, but not for the anti-R antibodies, where the index is close to 1. This suggests that additional C and T domain sites move to the trans face in the Lr" toxin but the location of at least some R domain sites do not change location. The increased movement of sites to the trans face in the Lr" toxin confirms and amplifies previous observations of increased toxin movement to the trans surface of the membrane in the Lr" conformation (5) and supports the role of C chain unfolding in translocation (5, 19) (see “Discussion”).
nonspecific antibody binding to the vesicles. As expected in a sample with vesicle-entrapped toxin, pelleted toxin is observed (lane 4 in each panel, lower half), but with externally added toxin, even the toxin does not pellet (lane 1 in each panel, lower half) because it does not bind to the vesicles.

In contrast, when toxin was inserted by exposure to low pH, the antibodies bound to the vesicles both with externally added (lanes 2 and 3 in each panel) and entrapped toxin (lanes 5 and 6 in each panel), in agreement with the ELISA results. This confirms the conclusion that there is translocation of sites on all three domains of the toxin to the trans face upon insertion.7

DISCUSSION

Structural Model For Membrane-inserted Diphtheria Toxin—This study shows that antibodies can be used to examine the structure of membrane-inserted diphtheria toxin. The observation that all three domains of the toxin undergo translocation to the trans face of the membrane requires a model of translocation in which all three domains participate in the insertion and translocation process in vitro (but perhaps not in vivo, see below). Despite the prejudice that the T domain, with its potentially transmembrane α-helices, should be the main part of the toxin undergoing insertion, the observation that isolated C (3, 6) and R domains (Ref. 10 and data not shown) become hydrophobic at low pH, isolated C domain can membrane insert (3) and proteolytic evidence for R domain insertion (23) support a role for these domains in the insertion and translocation process. Since the C and R domains lack long hydrophobic stretches, this brings up the question of the structure of membrane-inserted C and R domains. We have proposed that transmembrane β-sheets, similar to those formed by porins (20), may be formed by these domains (21). β-Strand 4 in the R domain is rich in hydrophobic residues, while β-strands 8 and 10 in the R domain and β-strands 4, 5, 6, and 8 in the C domain have the striking regions of alternating hydrophilic and hydrophobic residues found in transmembrane β-strands. An alternate possibility is that these strands insert in the membrane in a non-transmembrane fashion.

It has been observed that isolated catalytic, transmembrane, and receptor binding domains of Pseudomonas exotoxin A become hydrophobic at low pH (22). This may mean that the translocation mechanism for diphtheria toxin and exotoxin A is similar. This is interesting in terms of the similar secondary structure, but relative lack of sequence similarity, between the transmembrane and receptor binding domains of diphtheria toxin and Pseudomonas exotoxin A.

Unfolding of the C Domain and Translocation of Sites to the Trans Surface—There is an increasing body of evidence that the partial unfolding of the C domain promotes its translocation (5, 23, 24). Jiang et al. (5) showed that upon the unfolding of the C domain at 37 °C, additional regions of the A and B chain become exposed to the trans side of membrane-inserted toxin, suggesting translocation of both chains across a membrane bilayer. The observations in this report showing increased movement to the trans surface for toxin in the Lr0 conformation support this proposal, and indicate that the transmembrane insertion of some part of the T domain is also promoted by C domain unfolding. A model of toxin translocation is shown in Fig. 4. It shows that the C domain and certain regions of the T domain translocate across the bilayer upon unfolding of the C domain by heating to 37 °C, while the R domain does not translocate across the bilayer. Since our anti-T antibodies are against the amino-terminal end of the T domain, it suggests that some of the amino-terminal end of the T domain translocates across the bilayer upon C domain unfolding. This proposal is supported by protease and mutagenesis studies, which show that the amino-terminal end of the T domain is important for translocation of the toxin across a membrane (25–27). The observation that this change does not occur for the R domain suggests the R domain may not change its degree of insertion during the translocation process. In future studies it will be interesting to see how the exposure of T and R domains to the trans surface changes upon the release of the C domain from the membrane.

Orientation of Membrane-inserted Toxin in vivo—The observation that diphtheria toxin inserts in a mixed orientation brings up the question of whether this can occur in vivo. This cannot be answered at present, but it should be noted that several studies suggest this possibility. Two studies of toxin interaction with cells have been interpreted as indicating the presence of two populations of toxin, only one of which translocates (28, 29). In entry via endosomes it has been reported that only a third of the toxins translocated (29), which agrees with the percent toxin penetration to the trans surface found in this study. Another study has shown that not all toxin molecules insert such that they are accessible to a specific chemical modification (30). This could be interpreted in terms of toxin oligomers with mixed orientations. Nevertheless, it should be remembered the mixture of orientations (specifically the minor (30%) orientation) may not occur in vivo.
Acknowledgment—We thank Juanita Sharpe for developing the antibody-based assay for FITC-dextran leakage from vesicles.

REFERENCES
1. Choe, S., Bennett, M. J., Fuji, G., Curmi, P. M. G., Kantardjieff, K. A., Collier, R. J., and Eisenburg, D. (1992) Nature 357, 216–222
2. London, E. (1992) Biochim. Biophys. Acta 1113, 25–51
3. Zhao, J.-M., and London, E. (1992) J. Biol. Chem. 267, 15369–15377
4. Jiang, J., Abrams, F. S., and London, E. (1991) Biochemistry 30, 3857–3864
5. Jiang, J., Chung, L., and London, E. (1991) J. Biol. Chem. 266, 24003–24010
6. Montecucco, C., Schiavo, G., and Tomasi, M. (1985) Biochem. J. 231, 123–128
7. Papini, E., Schiavo, G., Tomasi, M., Colombatti, M., Rappuoli, R., and Montecucco, C. (1987) Eur. J. Biochem. 169, 637–644
8. Zalman, L. S., and Wisnieski, B. J. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 3341–3395
9. Hu, V. W., and Holmes, R. K. (1984) J. Biol. Chem. 259, 12226–12233
10. Espensen, Q. Y., Falnes, P. O., Olsnes, S., and Madshus, I. H. (1993) Biochem. J. 294, 663–666
11. Gonzalez, J. E., and Wisnieski, B. J. (1988) J. Biol. Chem. 263, 15257–15259
12. Tortorella, D., Sesardic, D., Dawes, D. S., and London, E. (1995) J. Biol. Chem. 270, 27439–27445
13. Tortorella, D., Ulbrandt, N. D., and London, E. (1993) Biochemistry 32, 9181–9188
14. Kagan, B. L., Finkelstein, A., and Colombini, M. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 4950–4954
15. Donovan, J. J., Simon, M. I., Draper, R. K., and Montal, M. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 172–176
16. Jiang, G., Solow, R., and Hu, V. W. (1989) J. Biol. Chem. 264, 13424–13429
17. Colman, P. M., Laver, W. G., Varghese, J. N., Baker, A. T., Tulloch, P. A., Air, G. M., and Webster, R. G. (1987) Nature 326, 358–363
18. Jin, L., Fieldy, B. M., and Wells, J. A. (1992) J. Biol. Chem. 266, 851–865
19. Falnes, P. O., Choe, S., Madshus, I. H., Wilson, B. A., and Olsnes, S. (1993) J. Biol. Chem. 269, 8402–8407
20. Weiss, M. S., Abele U., Wette J., Schiltz, E., and Schulz, G. E. (1991) Science 254, 1627–1630
21. London, E. (1992) Mol. Microbiol. 6, 3277–3282
22. Idziorek, T., FitzGerald, D., and Pastan, I. (1990) Infect. Immun. 58, 1415–1420
23. Moskaug, J. O., Stenmark, H., and Olsnes, S. (1991) J. Biol. Chem. 266, 2652–2659
24. Wiedlocha, A., Madshus, I. H., Mach, H., Middaugh, C. R., and Olsnes, S. (1992) EMBO J. 13, 4835–4842
25. vanderSpek, J. C., Mindell, J. A., Finkelstein, A., and Murphy, J. R. (1993) J. Biol. Chem. 268, 12077–12082
26. Madshus, I. H., Wiedlocha, A., and Sandvig, K. (1994) J. Biol. Chem. 269, 4648–4652
27. Madshus, I. H. (1994) J. Biol. Chem. 269, 17723–17739
28. Moskaug, J. O., Sandvig, K., and Olsnes, S. (1987) J. Biol. Chem. 262, 10339–10345
29. Papini, E., Rappuoli, R., Murgia, M., and Montecucco, C. (1993) J. Biol. Chem. 268, 1567–1574
30. Mindell, J. A., Zhan, H., Huynh, P. D., Collier, R. J., and Finkelstein, A. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 5272–5276
Immunological Analysis Shows All Three Domains of Diphtheria Toxin Penetrate across Model Membranes
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J. Biol. Chem. 1995, 270:27446-27452.
doi: 10.1074/jbc.270.46.27446

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