Hemolytically active, $^{125}$I-labeled $\Delta$-toxin from Clostridium perfringens was used to study the binding of this cytolytic to sheep, goat, human, rabbit, mouse, and guinea pig erythrocytes. The extent of toxin binding was correlated with the known hemolytic specificity of the toxin. Detailed studies of the binding were carried out on sheep erythrocytes which showed the highest sensitivity to lysis by $\Delta$-toxin. Simultaneous determination of toxin binding and release of intracellular $^{86}$Rb and hemoglobin suggested that toxin binding and membrane damage were separate sequential events. Toxin binding was rapid (2-5 min) and temperature-dependent. The extent of binding was temperature-independent. Binding was saturable, specific, relatively tight ($K_D = 4.4 \times 10^{-10} \text{ M}^{-1}$) and largely irreversible. A single type of binding site (7,000/sheep erythrocyte) was found. Cell-bound toxin was extractable by chaotropic ions. Preincubation of the toxin with N-acetylgalactosaminyl-[N-acetyleneuraminyl]-galactosylglucosylceramide (GM$_2$ ganglioside) inhibited both binding and hemolysis. Toxin binding was affected by pretreatment of sheep erythrocytes with pronase but not with trypsin or chymotrypsin. Cell treatment with neuraminidase prevented toxin binding by 30%. Preincubation of the toxin with specific immune sera blocked its binding on target cells. It is suggested that GM$_2$ ganglioside, a more complex membrane component containing this glycolipid or a structurally related molecule is the binding site for $\Delta$-toxin on the surface of sensitive erythrocytes.

There is a multifaceted interest in the study of cytolytic bacterial protein toxins ranging from their potential pathogenic effect in microbial infection to their use as molecular tools for probing membrane structure-function relationships (1-4). Our interest in this field of toxicological research prompted us to initiate the purification and characterization of Clostridium perfringens $\Delta$-toxin a few years ago (5) with the aim of investigating its membrane-damaging properties. This extracellular toxin is released from the cell along with bacterial protein toxins ranging from their potential pathogenic effect in microbial infection to their use as molecular tools for probing membrane structure-function relationships (1-4).

Materials and Methods

**Chemicals**—Carrier-free $^{125}$I-10% (6.65 mCi/mg of I) and $^{86}$RbCl (10 mCi/mg of Rb) were purchased from the Radiochemical Center, Amersham France (Versailles); bovine serum albumin from Biomeérieux (Lyon); bovine serum albumin from Sigma, trypsin (EC 3.4.21.4), and cy-chymotrypsin (EC 3.4.21.1) from Worthington.

**Buffers**—(a) isotonic HBS pH 8.0 (12); (b) 0.05 M TFB, pH 8.0 or 7.5, containing 0.158 M NaCl, (c) isotonic 0.15 M phosphate-buffered saline, pH 6.8 (Na$_2$HPO$_4$, 12H$_2$O, 13.42 g; NaH$_2$PO$_4$, 5.17 g; NaCl 4.5 g in 1 liter of distilled water).

**Sheep Red Blood Cell Suspension**—Erythrocyte suspension (6 x $10^8$ cells/ml) was prepared and standardized as described (12).

**Red Blood Cell Suspensions of Other Species**—Erythrocyte suspensions from the animal species listed in Table V were prepared and adjusted to the same hemoglobin content as that of standard SRBC (12). Stromata were prepared according to Fairbanks et al. (13).

*This laboratory is supported by Grant ERA 794 from the Centre National de la Recherche Scientifique. This work was supported in part by a Grant 77.7.1264 from the Delegation Générale à la Recherche Scientifique et Technique. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom all correspondence should be addressed.

The abbreviations used are: GM$_2$, N-acetylgalactosaminyl-[N-acetyleneuraminyl]-galactosylglucosylceramide; GM$_{1 b}$, galactosyl-N-acetylgalactosaminyl-[N-acetyleneuraminyl]-galactosylglucosylceramide; GM$_{1 a}$, N-acetyleneuraminylgalactosyl-N-acetylgalactosaminyl-[N-acetyleneuraminyl]-galactosylglucosylceramide; BBS, borate-buffered saline; TFB, Tris-formic acid buffer; HU, hemolytic unit; SRBC, sheep red blood cells; BSA, bovine serum albumin.
Clostridial Δ-Toxin Binding

**Δ-Toxin Preparation**—Highly purified Δ-toxin (M, = 42,000) was prepared as described (12). The protein was dissolved in washed SRBC suspension. One HU is that amount of toxin needed to release the hemoglobin from 50% of SRBC suspension at 37 °C within 45 min. The specific activity of the purified toxin was 325,000 HU/mg of protein. One HU is equivalent to 3 ng (7.2 x 10⁻⁸ mmol) of protein.

**Preparation of 125I-Δ-Toxin**—Purified toxin (equal to 12,000 HU corresponding to 10⁻⁷ mg) in 250 ml of TBF, pH 7.5, was labeled according to Greenwood et al. (14) with 0.5 mCi of ¹²⁵I Na. A volume of 200 μl (200 μg/ml) of chloramine-T solution was added dropwise. After the desired reaction time (10 min) at 4 °C, 250 μl of sodium metabisulfite (200 μg/ml) were introduced to stop the reaction. The mixture was then immediately transferred to a Bio-Gel P4 (Bio-Rad Laboratories, Inc., Richmond, CA) column (15,18 cm) previously equilibrated with 0.1% (v/v) BSA in TBF to avoid toxin adsorption on the gel. Labeled toxin emerged in the void volume and retained 90 to 95% of initial hemolytic activity. The labeled toxin pool (~1000 HU/ml) was distributed into 1 ml fractions in small glass tubes and stored at −18 °C. Labeled samples were used within 3 weeks. The radioactive specific activity of ¹²⁵I-Δ-toxin ranged from 2 to 3 cpm/μg of protein. For some experiments, higher amounts of labeled toxin were required. Concentrated toxin (50,000 HU) in 250 ml of TBF was labeled. The collected pool after gel filtration had a titer of 7,000 HU/ml (specific activity = 0.2 cpm/μg of protein).

**Results**

**Toxin Binding by Sheep Erythrocytes**—The binding of Δ-toxin by SRBC was evidenced from the protein pattern of washed stromata separated after lysis by the toxin and submitted to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Fig. 1). A toxin band was found at the same Rf as that of a free toxin sample. Autoradiography did not reveal any bands additional to that found at the Rf of the toxin indicating that the toxin remained intact after cell lysis.

**Binding Kinetics** of ¹²⁵I-labeled Δ-toxin (1 HU/3 × 10⁶ cells) at 37 °C and 0 °C is shown in Fig. 2. Binding occurred within a few minutes and was temperature-dependent. A second order rate constant, k₂, could be calculated from the data (10⁻⁷ M⁻¹·s⁻¹ at 37 °C). In contrast, the amount of bound toxin was temperature-independent (~35% of total counts).

Labeled toxin bound to the same extent to SRBC stromata produced by osmotic shock according to Fairbanks et al. (13) or by treatment with 50 HU of the sulfhydryl-dependent cytolysin, streptolysin 0 prepared in this laboratory (4).

The kinetics of hemoglobin and ⁸⁶Rb⁺ release (Fig. 3) indicated that cell lysis developed progressively after binding, suggesting a two-step process of toxin interaction with target cells. The rate of lysis was markedly influenced by temperature (data not shown).

**Specificity of Binding**—Pretreatment of SRBC with increasing amounts of native toxin prevented the binding of labeled toxin proportionately. Approximately, 85% of radio-

---

**Fig. 1.** Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of sheep erythrocyte stromata after hypotonic or toxin-induced lysis. Left, A mixture of ¹²⁵I-labeled Δ-toxin and unlabeled Δ-toxin (30 μg; 50,000 cpm) in TBF buffer containing 0.1% BSA, SRBC (7.5 × 10⁶ cells) lysed by osmotic shock and washed five times. C, SRBC (7.5 × 10⁶ cells) lysed with a mixture of labeled and unlabeled toxin (75 μg; 50,000 cpm). Right, autoradiogram of the gel slab of A, B, and C preparations.

---

**Enzyme Treatment of Sheep Erythrocytes**—Suspensions of SRBC were treated at 37 °C with occasional shaking with pronase (0.1 mg/ml of BBS, pH 7.8, 7 h), trypsin (0.2 mg/ml of BBS, pH 7.5, 15 h), chymotrypsin (0.2 mg/ml of BBS, pH 7.5, 15 h), V. cholerae neuraminidase (15 μg/ml of BBS, pH 6.8, 15 h) and C. perfringens neuraminidase (2 μg/ml of PBS, pH 6.8, 18 h), according to Maharan and Packrell (15). Trypsin was pretreated with N-tosyl-L-lysine chloromethyl ketone to remove traces of α-chymotrypsin. Chymotrypsin was treated with N-p-tosyl-L-lysine chloromethyl ketone to remove traces of chymotrypsin. Enzyme-treated erythrocytes were washed three times with BBS, pH 8.0, and then suspended in this buffer to 6 × 10⁶ cells/ml.

---

**Slab Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis**—Stratoma obtained after cytolysis with Δ-toxin were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis using a modification (12) of the method of Laemmli (16). Autoradiography was performed on the wet gel slab and exposure of the film (Kodak R-X-omat) was made for 24 h at room temperature.
Indicated times, 10 ng) was added to incubated at 25 °C, 37 °C (A—A). At indicated times, 10 ml of ice-cold BBS were added to stop the reaction and the mixtures were immediately centrifuged as described under "Materials and Methods." The percentage of hemoglobin released was correlated by the percentage of SRBC lysed by direct erythrocyte count in a hemocytometer indicating that lysis was an all-or-none phenomenon.

The release of intracellular 36Rb+(Fig. 2, inset) was measured as described in Fig. 2 for binding assay (A—A). The release of intracellular 36Rb+(Fig. 2, inset) and hemoglobin (□—□) was determined as described under "Materials and Methods." The percentage of hemoglobin released was correlated by the percentage of SRBC lysed by direct erythrocyte count in a hemocytometer indicating that lysis was an all-or-none phenomenon.

Activity associated with erythrocytes was attributable to specific binding (Fig. 4). This result is consistent with the finding that iodination did not modify the hemolytic titer of the toxin. No significant binding was observed with a toxin preparation previously heated for 45 min at 56 °C.

**Relationship Between Binding Capacity and Toxin or Cell Concentration**—No saturation of the erythrocytes by ∆-toxin was observed over a range of 1 to 200 HU (~3 to 600 ng) of 125I-labeled toxin incubated with 3 x 10^8 SRBC. In all instances, the specific binding was practically constant; 30-35% of the total amount of added toxin was found associated to the stromata recovered by centrifugation after incubation with ∆-toxin. Unbound toxin fraction present in the supernatant fluid of cell lysate was incubated with a fresh suspension of 3 x 10^8 SRBC. About 30% of the radioactivity was again found associated to the cells, indicating the homogeneity of activity associated with erythrocytes was attributable to specific binding (Fig. 4). This result is consistent with the finding that iodination did not modify the hemolytic titer of the toxin. No significant binding was observed with a toxin preparation previously heated for 45 min at 56 °C.

**Fig. 2.** Effect of temperature on the kinetics of binding of 125I-labeled ∆-toxin by sheep erythrocytes. One HU of toxin (3 ng) was added to 3 x 10^8 SRBC to a final volume of 1.5 ml and incubated at 3 °C (■), 25 °C (□—□), and 37 °C (A—A). At indicated times, 10 ml of ice-cold BBS were added to stop the reaction and the mixtures were immediately centrifuged as described under "Materials and Methods."

**Fig. 3.** Time course of toxin binding by sheep erythrocytes and release of 36Rb+ and hemoglobin. One HU (3 ng) of 125I-labeled toxin was added to 3 x 10^8 SRBC to a final volume of 1.5 ml. At indicated times, samples were treated as described in Fig. 2 for binding assay (A—A). The release of intracellular 36Rb+(Fig. 3, inset) and hemoglobin (□—□) was determined as described under "Materials and Methods." The percentage of hemoglobin released was correlated by the percentage of SRBC lysed by direct erythrocyte count in a hemocytometer indicating that lysis was an all-or-none phenomenon.

**Fig. 4.** Inhibition of 125I-labeled ∆-toxin binding by native ∆-toxin. Increasing amounts of unlabeled ∆-toxin (3 µg to 60 µg) were added into tubes containing 3 x 10^8 SRBC to give a final volume of 1.5 ml. The mixtures were incubated at 25 °C for 10 min and then 10 HU (30 ng) of labeled toxin (1.8 x 10^3 cpm/µg of protein) were added into each tube. After incubation for 10 min, specific binding to cell pellets was determined as described under "Materials and Methods." The percentage of hemoglobin released was correlated by the percentage of SRBC lysed by direct erythrocyte count in a hemocytometer indicating that lysis was an all-or-none phenomenon.

The effect of erythrocyte concentration on binding was investigated by incubation of 17 HU (50 ng) of labeled toxin with varying amounts of SRBC. As shown in Fig. 5, toxin binding increased proportionally to cell concentration until an excess of SRBC was reached. The maximum amount of toxin bound was 57% of total toxin added. Total lysis was observed (Fig. 5, inset) up to 6 x 10^8 cells.

On the basis of these data it appeared interesting to investigate toxin binding over a wide toxin concentration range. 125I-∆-Toxin (60 to 1600 HU) was thus incubated with 6 x 10^7 SRBC. Under these conditions, the specific binding of the toxin was concentration-dependent and saturable (Fig. 6). Scatchard analysis of the binding curve gave a straight line (Fig. 6, inset). The apparent association constant was K_a ~ 4.4 x 10^6 M^-1 at 37 °C and the number of apparent binding sites calculated from the intercept with the ordinate was ~7000/cell.

**Release of Membrane-bound Toxin**—About 15% of the toxin bound by SRBC suspension was progressively (and spontaneously) released by the ghosts incubated at 37 °C in BBS, pH 8.0, over 88 h (Fig. 7). Incubation in the presence of a large excess of unlabeled toxin did not show significantly higher release. This finding suggests that the ∆-toxin was firmly associated with the membrane and was not appreciably displaced by incubation with free toxin.

**Susceptibility of Membrane-bound Toxin to Elution by**
Chaotropic Salts or Detergents—It has been reported (18, 19) that certain anions such as the haloacetates, thiocyanates, and, to a lesser extent, other salts act as chaotropic ions tending to disorder water structure, thereby destabilizing membrane organization. Guanidine HCl and urea also behave like chaotropic agents. The tendency of these components to decrease membrane stability has been assigned to the following order (18, 20): guanidine, SCN− > I− > Br− > Cl− > HPO₄²⁻ > CH₃COO−. It appeared, therefore, interesting to investigate whether such reagents may remove membrane-bound Δ-toxin from SRBC stromata lysed with the toxin. As shown in Table I, Δ-toxin was almost entirely extracted by 8 M guanidine HCl or urea, 3 M KSCN and KI. The other salts of weaker chaotropic effects were less effective. However, antichaotropes such as PO₄³⁻ ions, which increase the structural order of water thereby enhancing hydrophobic associations, extracted 30% of bound toxin. A similar result was also found by treating the stromata with isotonic 0.2 M BBS buffer, pH 8.0, as well as with 0.5 M Triton X-100.

Effect of Enzyme Treatment of Toxin and Cells on Toxin Binding and Release—Binding capacity and hemolytic activity of ¹²⁵I-Δ-toxin were completely abolished by preincubation of toxin preparation (50 HU, 37 °C, 1 h) with pronase (5 μg), chymotrypsin (10 μg), or trypsin (10 μg). The effect on the binding of the same amount of labeled toxin on SRBC (3 × 10⁹ cells) previously treated with these proteases and with neuraminidases was investigated. Pretreatment of cells with trypsin and chymotrypsin did not interfere with either toxin binding or lysis. In contrast, pronase pretreatment inhibited almost completely (95%) toxin binding. Cell treatment by C. perfringens neuraminidase inhibited toxin binding by 30%. In contrast, no inhibition was found by treatment with V. cholerae neuraminidase although the substrates of this enzyme were hydrolyzed as evidenced by the liberation of N-acetylmuramic acid determined by the thioribarbituric acid assay according to Warren (21). The effect of the same enzymes on
TABLE II

Release of labeled material from 125I-labeled Δ-toxin bound to sheep erythrocytes treated with various enzymes

Same legend as Table I except for incubation with enzyme solutions which was performed overnight at 37 °C (enzyme concentration is that used for SRBC treatment as mentioned under "Materials and Methods").

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Cell-bound toxin released %</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (BBS, pH 8.0)</td>
<td>20</td>
</tr>
<tr>
<td>Trypsin</td>
<td>31</td>
</tr>
<tr>
<td>Chymotrypsin</td>
<td>47</td>
</tr>
<tr>
<td>Pronase</td>
<td>38</td>
</tr>
<tr>
<td>V. cholerae neuraminidase</td>
<td>20</td>
</tr>
<tr>
<td>C. perfringens neuraminidase</td>
<td>44</td>
</tr>
</tbody>
</table>

The hemolytic activity of control was considered to be 100%.

The release of radioactive membrane-bound toxin is shown in Table II. Only chymotrypsin, C. perfringens neuraminidase and pronase liberated significant radioactive material from the ghosts as compared to control.

Influence of Gangliosides and Other Lipids on 125I-Δ-Toxin Binding—We have previously shown that Gm2 ganglioside inhibited significantly SRBC lysis by Δ-toxin (12). Other gangliosides were much less inhibitory whereas cholesteryl, egg lecithin, and sphingomyelin were inactive. As shown in Table III, inhibition of toxin binding was observed for low concentrations of Gm2 ganglioside and higher concentrations of G1, G2, Gm1, Gm2, Gm3, and Gm4 gangliosides. The other lipids tested did not interfere with binding. Binding and hemolytic activity were significantly correlated.

Effect of Antibodies, Ca2+, and Tris on Toxin Binding—An immune serum from a rabbit immunized with pure Δ-toxin (12) containing 160 neutralizing units/ml prevented the fixation of 125I-Δ-toxin by 85% over a concentration range of 5 to 100 HU (Table IV). No hemolysis was observed at any concentration. The fraction of toxin material which remained associated with the cells is very likely due to nonspecific fixation of the labeled preparation. This finding is consistent with the data found for nonspecific binding of the toxin in the absence of antiserum. In similar experiments, no inhibition of binding was observed with 10 mM Ca2+ ions and 50 mM Tris which have been previously shown to completely inhibit the hemolysis of SRBC by Δ-toxin (5).

Effect of antitoxin serum on toxin binding on sheep erythrocytes

Varying amounts of 125I-labeled Δ-toxin (5 to 100 HU) were incubated with 50 μl of undiluted rabbit serum raised against purified Δ-toxin (12). This quantity of immune serum neutralizes about 190 HU. Toxin-antitoxin mixtures were adjusted to a final volume of 1 ml and incubated at 37 °C for 10 min. The mixture was then tested on SRBC as described under "Binding Assay." Undiluted normal rabbit serum did not significantly affect toxin binding.

TABLE IV

Effect of antitoxin serum on toxin binding on sheep erythrocytes

<table>
<thead>
<tr>
<th>125I-toxin bound</th>
<th>125I-toxin-antitoxin mixture bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>cpm</td>
<td>cpm</td>
</tr>
<tr>
<td>1,515</td>
<td>260</td>
</tr>
<tr>
<td>3,150</td>
<td>449</td>
</tr>
<tr>
<td>17,559</td>
<td>2,917</td>
</tr>
<tr>
<td>33,409</td>
<td>5,123</td>
</tr>
</tbody>
</table>

* With respect to the data of the first column.

The kinetics of toxin binding was studied in parallel with that of the release of hemoglobin and intracellular "Rb" from erythrocytes preloaded with this radioactive marker which is a K+ ion analogue commonly used as an indicator of cell damage in membrane studies (22). Toxin-induced cytolyis appeared as a sequential process characterized by (i) rapid binding even at 0 °C and measurably faster at 37 °C, (ii) a slower and markedly temperature-dependent process leading to irreversible permeability changes evidenced by the leakage of internal components of target cells (Fig. 3). However, the extent of binding for a given concentration of toxin was temperature-independent. The events taking place during the lag between binding and marker efflux remain to be analyzed. It is worth noting that the action of many cytolytic and noncytolytic toxins is similarly characterized by a relatively rapid time-dependent binding with subsequent slower time-dependent induction of relevant biological effect (23-26).

Toxin binding was specific to a large degree (Fig. 4). The percentage of specific binding remained constant (~30-35%) within a concentration range of 1 to 200 HU/3 × 10^5 cells. This result may be due to increasing unmasking of toxin-binding sites resulting from membrane disorganization during the lytic process. Under these conditions no evaluation of the number of binding sites is feasible. However, by using higher amounts of toxin (Fig. 6), specific binding was saturable. The calculated association constant appeared relatively tight (Kd ~ 4.4 × 10^6 M^-1). A single type of binding site was inferred from the Scatchard plot. About 7,000 molecules were bound per sheep erythrocyte. In a similar study, 5,000 binding sites
per rabbit erythrocyte were found for staphylococcal α-toxin (23). Toxin binding was practically irreversible. A small fraction of bound toxin (~15%, very likely corresponding to that non-specifically bound) dissociated at a very slow rate (over 88 h) from the erythrocytes suspended in either buffer or an excess of unlabeled toxin. Similar findings were reported for the release rate of staphylococcal α-toxin by erythrocytes (23) and C. perfringens enterotoxin by rabbit intestinal cells (27). The almost total irreversibility of toxin binding in these systems and in that studied here may be due to either conformational changes of bound toxin or eventual polymerization induced by membrane lipids as reported for other bacterial cytolsins such as sulfhydryl-activated toxins (28) and staphylococcal α-toxin (29, 30).

Toxin binding increased proportionally with cell concentration until an excess of cells was reached (Fig. 5). Bound toxin was dissociated by incubation of cell ghosts with chaotropic ions whereas these agents effected no release of SH-activated toxins (4) which are known to form strong hydrophobic complexes with bilayer cholesterol (1, 4, 28). This finding is compatible with the hypothesis that Δ-toxin remains associated with the cell surface after binding without significant insertion into the bilayer. This hypothesis appears to be substantiated by the absence of the labeling of cell-bound toxin by the photoactivatable glycolipid probe 12-(4-azido-2-nitrophenoxy)-stearoyl-[1-14C]glucosamine (31) which inserts into bilayers as reported for cholera toxin (32), complement (33), and recently for the SH-activated toxin alveolysin (31).

Significant correlation between the binding capacity of erythrocytes from seven mammalian species and their sensitivity to lysis by Δ-toxin is shown in Table V. However, both parameters were not proportionally correlated, indicating that other factor(s) besides binding capacity may be involved in the lytic process.

Toxin binding by SRBC was inhibited by preincubation of Δ-toxin with specific antitoxin immune serum (Table IV). Gm2 ganglioside (Table III) inhibited rather preferentially and at a relatively low concentration both binding and cell lysis (see also Ref. 12), as compared to other gangliosides and other lipids. In addition the binding of 1 nmoi of 125I-Δ-toxin by SRBC was not affected by preincubation of these cells with 200 nmoi of cholera toxin which is known to bind G0s but not Gm2 ganglioside. This suggests that the latter ganglioside, a more complex membrane component containing this glycolipid or a structurally related molecule, is involved in Δ-toxin binding. The fact that preincubation of sheep erythrocytes with C. perfringens neuraminidase, known to catalyze the hydrolysis of N-acetylneuraminic acid from Gm2 (34), decreased binding by 30% favors this hypothesis. In contrast, V. cholerae neuraminidase did not affect binding in spite of N-acetylneuraminic acid release from the cells. This enzyme hydrolizes sialoglycoproteins but is inactive or poorly active on Gm2 and Gm4 gangliosides due to steric hindrance (34, 35). Pretreatment of erythrocytes with trypsin and chymotrypsin did not affect binding in contrast to pretreatment with pronase. This enzyme has been reported to cleave only two polypeptides (band 3 and glycoporin) on the erythrocyte membrane whereas trypsin specifically degraded glycoporin, leaving band 3 and other polypeptides intact (see Ref. 15 for references). Membrane carbohydrates are also released by pronase (36). Whether the wide variability in binding capacity of erythrocytes from different species is correlated to Gm2 content and/or its availability to toxin in cell membranes is not known. Also the content and distribution of gangliosides including Gm2 in erythrocyte membranes are still poorly documented (37-41). The analytical data for other tissues have always shown that Gm2 is a minor component in eukaryotic membranes as compared to Gm1 and other gangliosides (37). This observation is consistent with the apparently lower number of binding sites on sheep erythrocytes. It is worth noting that different gangliosides have been reported to function as receptors for various bacterial toxins, several glycoproteins, hormones, interferon, and other bioactive factors (see Refs 12 and 41 for references).

To our knowledge, the effect of Δ-toxin on cells other than erythrocytes has not been investigated so far. Recently, we have initiated such a study and found that rabbit macrophages and lymphocytes, platelets from various species (man, mouse, rabbit, guinea pig) as well as Xenopus laevis oocytes (kindly provided by P. Bouquet) were lysed or damaged by purified Δ-toxin (manuscript in preparation). These findings indicate that the insensitivity of the erythrocytes from the mentioned species was not due to the species per se but rather to the lack of masking of toxin receptors which are apparently present on the membrane of other cell types. Also, human fibroblasts exposed to our toxin preparation are damaged as evidenced by the release of entrapped low molecular marker (42).

Acknowledgments—We are grateful to Dr. J. H. Freer, University of Glasgow, for stimulating discussion and review of the manuscript prior to its submission and to Dr. F. Modabber, Pasteur Institute, for helpful advice. We thank Monique Agougué for technical assistance.

REFERENCES
11. Orleans, E. S., and Jones, V. E. (1958) Immunology 1, 296-290

TABLE V
Binding of [125I]Δ-toxin to erythrocytes of various species

<table>
<thead>
<tr>
<th>Species</th>
<th>Specific [125I] toxin bound</th>
<th>Relative sensitivity to lysis</th>
<th>R**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep</td>
<td>79,900 100%</td>
<td>100%</td>
<td>1</td>
</tr>
<tr>
<td>Goat</td>
<td>69,960 87%</td>
<td>25%</td>
<td>3.48</td>
</tr>
<tr>
<td>Human</td>
<td>11,985 15%</td>
<td>0.04</td>
<td>375</td>
</tr>
<tr>
<td>Rabbit</td>
<td>3,525 4.4%</td>
<td>0.02</td>
<td>220</td>
</tr>
<tr>
<td>Horse</td>
<td>3,755 4.7%</td>
<td>0.015</td>
<td>313</td>
</tr>
<tr>
<td>Mouse</td>
<td>2,800 &lt;0.1%</td>
<td>0.015</td>
<td>2</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>2,180 2.7%</td>
<td>0.001</td>
<td>2700</td>
</tr>
</tbody>
</table>

* Relative percentage of specific binding with respect to counts/min bound on SRBC as 100%.
** R is the ratio of relative per cent binding to relative per cent hemolysis.

2 C. Jolivet-Reynaud and J. E. Alouf, manuscript in preparation.
Clostridial A-Toxin Binding

Binding of Clostridium perfringens 125I-labeled delta-toxin to erythrocytes.
C Jolivet-Reynaud and J E Alouf


Access the most updated version of this article at http://www.jbc.org/content/258/3/1871

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at
http://www.jbc.org/content/258/3/1871.full.html#ref-list-1