TABLE 1 Summary of native and heavy atom derivative data

	Native	CdCl ₂	K ₂ PtCl ₄	Trimethyl lead acetate	AgNO ₃	Mersalyl acid
Number of crystals	1	2	1	1	1	1
Total reflections	84,281	106,731	75,124	62,713	72,494	86,978
Complete (%)	98	98	85	85	71	93
<i>R</i> _{merge} (%) [*]	5.4	5.5	6.2	4.8	5.2	4.6
<i>R</i> _{deriv} (%) [†]	—	17.2	33.8	19.8	15.0	13.7
Number of sites	—	2	7	4	2	3
Phasing power [‡]	—	0.46	1.24	1.14	1.05	0.65

VT-1 was purified and crystallized as previously described by us¹⁵ and independently by Hart *et al.*¹⁶, except that addition of 1.5 mM zinc chloride to the crystallization medium increased the thickness of the crystals and improved their morphology. The crystals belong to space group P2₁2₁2₁ (cell dimensions *a*=59.6 Å, *b*=102.4 Å, *c*=56.1 Å) with one B-subunit pentamer per asymmetric unit. Data to 2.2 Å from native crystals (co-crystallized with zinc) and from five heavy-atom derivatives were collected using a San Diego-style multiwire counter area detector with rotating anode source. Heavy-atom sites were found using difference Patterson and difference Fourier analyses. Heavy-atom parameters were refined with a modified version of the program PHARE¹⁷ resulting in an overall figure of merit of 0.58 (2.5–2.5 Å). The amino-acid sequence of one B subunit monomer was fitted to an electron-density map calculated with unmodified multiple isomorphous replacement (MIR) phases using the program FRODO¹⁸, based on a skeletonized map produced with the program BONES¹⁹. The helical sequence (residues 36–46) was fitted in all five B monomers and the symmetry operations that superimposed these helices were used to generate the complete B pentamer. The model was improved by rigid-body refinement followed by simulated annealing with molecular dynamics using the program XPLOR²⁰ and then by alternate rounds of restrained parameter least-squares refinement with the program PROLSQ²¹ and model building with FRODO. Model phases were combined with the original MIR phases using the program SIGMAA²² during the initial rounds of refinement. The current model, which includes 2,700 protein atoms, 102 waters and 3 zinc atoms, has a crystallographic R-factor of 17.7% for all data between 10 and 2.2 Å. The r.m.s. deviation from ideal values for bond lengths is 0.017 Å and for bond angles is 2.9°. The r.m.s. deviation of atomic coordinates of main chain atoms is about 0.3 Å between monomer pairs 1–3, 1–4, 1–5, 3–4, 3–5 and 4–5, but about 0.4 Å between monomer pairs 2–1, 2–3, 2–4 and 2–5 (monomer numbering is described in the Fig. 1 legend).

$$^* R_{\text{merge}} = \sum_i |I_i - \langle I \rangle| / \sum_i I_i$$

$$^{\dagger} R_{\text{deriv}} = \sum_i |F_{\text{PH}} - |F_{\text{P}}|| / \sum_i |F_{\text{P}}|$$

$$^{\ddagger} \text{Phasing power} = [\sum_i |F_{\text{H}}|^2 / \sum_i (|F_{\text{PH}}(\text{obs})| - |F_{\text{PH}}(\text{calc})|)^2]^{1/2}$$

residues with polar and acidic side chains which could make hydrogen-bond interactions with polar groups of the carbohydrate, and contains exposed aromatic residues which could stack against the sugar rings. Site-directed mutagenesis has shown that at least two amino acids lining this cleft, Asp 17 and Lys 53, are involved in carbohydrate binding to Shiga toxin¹⁰. By contrast, loss of Gb₃ binding in two VT-2 mutants, Ala 43 → Thr and Gly 60 → Asp (ref. 11) is probably explained by poor folding of the protein; Ala 43 lies on a buried surface of the helix, and Gly 60 has a left-handed α-helical conformation in a turn. The proposed carbohydrate-binding site provides a structural explanation for the altered binding specificity associated with the double mutation Gln 65 → Glu, Lys 67 → Gln in VT-E (ref. 12). The mutant protein showed selective loss of Gb₄ binding with retained Gb₃ binding, consistent with binding of the terminal sugar residue of Gb₄ to the side chains of amino acids 65 and 67. These amino acids are found in the lower portion of the proposed carbohydrate-binding site (as it is shown in Fig. 2), suggesting that the glycolipid binds with the ceramide end (hence the cell membrane) at the upper extreme of the binding cleft. If the interaction between the A and B subunits of VT-1 is the same as in LT (ref. 2), with the A subunit lying below the B pentamer as it is shown in Fig. 2, the holotoxin would bind to cells with the A subunit away from the membrane. This agrees with the proposed membrane-binding orientation of LT and cholera toxin, based on the recently determined crystal structure of LT complexed with lactose¹³. □

- Seidah, N. G. *et al.* *J. Biol. Chem.* **261**, 13928–13931 (1986).
- Jaciewicz, M., Clausen, H., Nudelman, E., Donohue-Rolfe, A. & Keusch, G. T. *J. exp. Med.* **163**, 1391–1404 (1986).
- Lingwood, C. A. *et al.* *J. Biol. Chem.* **262**, 8834–8839 (1987).
- Waddell, T., Head, S., Petric, M., Cohen, A. & Lingwood, C. *Biochem. biophys. Res. Commun.* **152**, 674–679 (1988).
- DeGrandis, S., Law, H., Brunton, J., Gyles, C. & Lingwood, C. A. *J. Biol. Chem.* **264**, 12520–12525 (1989).
- Lindberg, A. A. *et al.* *J. Biol. Chem.* **262**, 1779–1785 (1987).
- Quiocho, F. A. *Pure appl. Chem.* **61**, 1293–1306 (1989).
- Jackson, M. P., Wadolkowski, E. A., Weinstein, D. L., Holmes, R. K. & O'Brien, A. D. *Bacteriology* **172**, 653–658 (1990).
- Perera, L. P., Samuel, J. E., Holmes, R. K. & O'Brien, D. J. *Bact.* **173**, 1151–1160 (1991).
- Tyrrell, G. J. *et al.* *Proc. natn. Acad. Sci. U.S.A.* (in the press).
- Sixma, T. K. *et al.* *Nature* **355**, 561–564 (1992).
- Bernstein, F. C. *et al.* *J. molec. Biol.* **112**, 535–542 (1977).
- Boothoo, A., Read, R. J. & Brunton, J. *J. molec. Biol.* **221**, 729–731 (1991).
- Hart, P. J. *et al.* *J. molec. Biol.* **218**, 691–694 (1991).
- Otwinski, Z. *Am. crystallogr. Ass. 1990 Annual Meeting, New Orleans Abstr.* C04 (1990).
- Jones, T. A. *J. appl. Crystallogr.* **11**, 268–272 (1978).
- Jones, T. A. & Thirup, S. *EMBO J.* **5**, 819–822 (1986).
- Brünger, A. T., Kuriyan, J. & Karplus, M. *Science* **235**, 458–460 (1987).
- Hendrickson, W. A. & Konert, J. H. In *Computing in Crystallography* (eds Diamond, R., Ramaseshan, S. & Verkatesan, K.) 13.01–13.23 (Indian Institute of Sciences, Bangalore, 1980).
- Read, R. J. *Acta crystallogr.* **A42**, 140–149 (1986).
- Kabsch, W. & Sander, C. *Biopolymers*, **22**, 2577–2637 (1983).
- Ramotar, K. *et al.* *Biochem. J.* **273**, 805–811 (1990).

ACKNOWLEDGEMENTS. This work was supported by the MRC of Canada (J.L.B., R.J.R. and P.E.S.), the Alberta Heritage Foundation for Medical Research, the Ontario Ministry of Health (G.J.T.) and by an award to R.J.R. from the Howard Hughes Medical Institute. The coordinates of the VT-1 B subunit have been deposited with the Brookhaven Protein Data Bank¹⁴ (entry name, 1BOV).

ERRATUM

Mouse *Small eye* results from mutations in a paired-like homeobox-containing gene

Robert E. Hill, Jack Favor, Brigid L. M. Hogan, Carl C. T. Ton, Grady F. Saunders, Isabel M. Hanson, Jane Prosser, Tim Jordan, Nicholas D. Hastie & Veronica van Heyningen

Nature **354**, 522–525 (1991).

As a result of an error in the *Nature* office, an uncorrected version of this letter appeared in the 19/26 December issue. In the legend to Fig. 3 and in Figs 3b, 3d and 4 the designation *Sey*^{IN} should read *Sey*^{Neu} as in the text, and a revised Fig. 3e is shown below.

The penultimate sentence in paragraph 2 should read “In the homozygous *Sey/Sey* embryo (Fig. 1c) no eyes are visible and the nasal cavities do not develop^{15,16} resulting in narrowed, imperforate snouts.” The penultimate sentence in paragraph 3 should read: “As the mouse homologue of the WT-1 (Wilm’s tumour) gene is also deleted (data not shown), the *Sey*^H deletion covers a large region.”

Received 16 October; accepted 17 December 1991.

- Brunton, J. L. in *The Bacteria* Vol. 11, *Molecular Basis of Bacterial Pathogenesis* (eds Iglewski, B. & Clark, V) 377–398 (Academic, New York, 1990).
- Sixma, T. K. *et al.* *Nature* **351**, 371–377 (1991).