diffusion across capillaries into the extravascular space[18]. This helps to explain why the concentration of NSILA in lymph is half that in plasma[19] whereas insulin (which has no binding protein) can equilibrate between these two compartments. The most intriguing feature of this binding protein is that its concentration in the plasma is regulated by GH[20,21] and the binding of NSILA to its binding protein prolongs NSILA's half-life in plasma from a few minutes to several hours. The release of NSILA from its binding protein is thus a major factor in governing the availability of this growth promoting hormone to the tissues of the body. This also explains why searches in various organs for high concentrations of NSILA were unsuccessful; NSILA is concentrated in one of the largest 'organs' of the body—the plasma.


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# Toxin subunits

*from a Correspondent*

DURING the past couple of years knowledge of the mechanism of action of bacterial toxins, particularly cholera, tetanus and diphtheria toxins, and studies of hormone action have pointed out several areas of similarity in these diverse biological systems. Apparently, similar surface membrane receptors may be part of the systems responsible for uptake of some toxins and hormones, an obligatory step in the biological action of these substances on cells. Perhaps the most striking result comes from L. D. Kohn and his collaborators who find some homologies in peptide sequences between the cholera toxin and thyroid-stimulating hormone (TSH). Mullin *et al. Proc. natn. Acad. Sci. U.S.A.* **73**, 842, 1679; 1976; Ledley et al. *Biochem. biophys. Res. Commun.* **69**, 852; 1976; Meldolesi *et al. Proc. natn. Acad. Sci. U.S.A.* **73**, 4060; 1976). For this reason alone, current research on bacterial toxin structure and action has wide implications.

Several recent papers provide detailed information on the structure of tetanus and diphtheria toxins. These two toxins superficially have very similar structures and in the native state consist of single polypeptide chains which are easily 'nicked' by proteases into two polypeptides held together by disulphide bonds. Both polypeptide 'subunits' are essential for the activity of either toxin on intact cells. One of the fragments of 'nicked' diphtheria toxin (A subunit), however, is active in inhibiting protein synthesis in cell-free extracts by inactivating elongation factor 2 (translocase) in precisely the same way as intact toxin exerts its killing effect on unbroken cells. The second polypeptide subunit of nicked diphtheria or tetanus toxin then is reasonably assumed to be required to attach the toxin to the surface membrane of whole cells. Until recently this point was hard to establish for diphtheria toxin, since the B subunit is insoluble under normal conditions after disulphide bond cleavage of nicked toxin and may be kept in soluble form only in urea or other protein denaturants. Two groups have now devised methods for the isolation of stable B fragments. Helting and Zwisler (*Behring Inst. Mitt.* No. 59, 92–101, 1976) treated diphtheria toxin with formaldehyde under mild conditions before trypsinisation and reduction of disulphide bonds. The B fragment was immunogenic in rabbits or guinea pigs and the specific antibodies elicited by immunisation blocked the lethal action of diphtheria toxin in animals or whole cells, presumably by interfering with the interaction of the appropriate peptide sequence of the toxin with specific surface membrane receptors. In contrast, these antibodies failed completely to affect the enzymatic activity of isolated fragment A in inhibiting *in vitro* protein synthesis. More direct evidence for fragment B interactions with cells was obtained by Everse *et al.* (*Proc. natn. Acad. Sci. U.S.A.*, **74**, 472; 1976). The chains were stabilised either by using low concentrations of sodium dodecyl sulphate or by sulphonation. Solutions containing B fragment protected HeLa cells from the effect of intact diphtheria toxin, while A fragment was completely inactive. Although rather large amounts of B fragment were required for protection against the toxin the effect does seem to be specific and strengthens the idea that the B fragment of the toxin molecule probably exerts the initial interaction with cell membrane receptors. Everse *et al.* also showed that 'unnicked' toxin was inactive when tested against HeLa cells under conditions where the 'nicked' toxin was highly cytotoxic. Probably, therefore, interaction of the toxin with cell surface receptors takes place most efficiently after 'nicking', a point that is interesting in the context of reports that some tumour cells are more sensitive to the action of diphtheria toxin than normal cells (Iglewski & Rittenberg *Proc. natn. Acad. Sci. U.S.A.*, **71**, 2707; 1974; Pappenheimer & Randall *Proc. natn. Acad. Sci. U.S.A.* **72**, 3149; 1975) and the induction of proteolytic enzymes in some transformed cell lines (see for example Chen & Buchanan *Proc. natn. Acad. Sci. U.S.A.* **72**, 1132; 1975).

The subunit of 'nicked' tetanus toxin analogous to fragment B of diphtheria toxin has also been identified recently (Helting & Zwisler *J. biol. Chem.* **252**, 187; 1977; Helting *et al. J. biol. Chem.* **252**, 194; 1977). The larger of the two polypeptides (heavy chain) derived after mild trypsinisation retained all of the receptor binding activity of the native toxin and a specific antiserum directed against a part of the heavy chain completely prevented activity of whole toxin. These new techniques, therefore, make available stable fragments of both diphtheria and tetanus toxin that are efficient in eliciting antibodies which interfere with the interactions with brain receptors and neutralise toxic action. Such vaccines may have advantages over the standard preparations against inactivated toxoids. Reactions of delayed hypersensitivity are known in immunisation, particularly of older children, for example (see Helting & Zwisler *op. cit.* 1976) and the use of peptide fragments lacking the antigenic determinants producing these undesirable responses would be a distinct advance. □

# Microtubes

*from Peter J. Smith*

EVERY now and then someone decides to investigate a terrestrial phenomenon which has been known for many decades, if not a hundred years or more, but which has never before been studied in any detail. Quite why certain phenomena should be ignored for so long is far from clear. Some have probably been considered trivial —of interest in themselves, perhaps,