were used, and a K_D of 6 nM found in striatum. Calculation of this in vitro K_D in terms of wet wt gives a K_D of 600 pmoles/g.) In the cerebellum, a negligable amount of saturable d-LSD binding was found. This allows the use of the cerebellum as an 'internal standard'[5] for the amount of unspecifically bound d-LSD. By calculating the ratio of the amount of specifically bound d-LSD in a given region to the amount of unspecifically bound d-LSD in the cerebellum, a correction for the experimental procedure and day-to-day fluctuations is obtained. This correction results in an interindividual variation coefficient of less than 10%, smaller than that obtained in vitro, and testifies to the remarkable reproducibility of the method.

The stereospecificity of d-LSD binding was demonstrated by the fact that specific binding could be reduced by the d- but not the l-enantiomer of unlabelled LSD injected simultaneously (figure 3), even at the relatively high dose of 150 µg/kg.

Preliminary studies of d-LSD displacement with a number of drugs were carried out using the maximum displacement found with 1 mg/kg cold d-LSD as 100%. The mean percent displacement for all regions was similar for a given drug. In striatum, neither apomorphine (5 mg/kg 60 min before ^{3}H-d-LSD; displacement 18±14% [SD, N=3]) nor 1–5HTP-ethylester (100 mg/kg 120 min before ^{3}H-d-LSD; displacement 5±13%) had a noticeable effect, whereas haloperidol (1.25 mg/kg 90 min before ^{3}H-d-LSD; displacement 63±9%) and methiothepin (20 mg/kg 120 min before ^{3}H-d-LSD; displacement 92±10%) had marked effects. Without extensive dose-response curves, no conclusion as to the relative efficacy of dopamine and/or serotonin agonist/antagonists on d-LSD binding can be drawn; however, such pharmacological studies in vivo, in particular by improving the dissection technique to measure d-LSD binding in specific dopaminergic or serotoninergic brain nuclei (in preparation) may provide more detailed information.

This method of d-LSD binding in vivo is therefore characterized by high affinity, stereospecificity and regional selectivity. It may be a useful adjunct to in vitro methods for measuring changes in turnover at the synaptic level. Its advantage over in vitro methods is the intact environment of the receptor at the time of injection.

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Bacillus thuringiensis δ-endotoxin: Evidence that toxin acts at the surface of susceptible cells

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Summary. Enzymically activated δ-endotoxin of *Bacillus thuringiensis* covalently bound to Sephadex beads, has the same effect on insect cells in tissue culture as free toxin. The effect is prevented by antitoxin antibody and heat denaturation and is not due to a nonspecific protein effect, the beads, or toxin released from the beads. The toxin, therefore, probably acts at the cell surface.

The insecticidal activity of *Bacillus thuringiensis* preparations resides principally in the proteinaceous parasporal inclusion (δ-endotoxin) formed as a concomitant of sporulation[1]. The parasporal body is dissolved in the insect gut to yield toxic fragments ranging in size from <5,000 to >100,000 daltons[2]; however, only atoxic dipeptide fragments of toxin are detected in hemolymph after ingestion of toxin[3]. The direct effect of the toxin appears to be limited to the epithelial cells of the midgut which suffer metabolic disturbances[4], apical swelling, and lysis[5]. Death of the insect appears to stem from leakage of gut contents into the hemocoel due to loss of integrity of the gut wall[6].

Toxin stimulates glucose uptake by midgut epithelium within 1 min after oral administration[4]. It does not, however, cause accumulation of Na^+, K^+, Ca^{++} or Mg^{++} ions[7] as had been suggested[2]. We recently showed that some insect cell lines in tissue culture are susceptible to the toxin with an LD_{50} of 0.06 mg/ml[8]. With immobilized toxin we could determine if the toxin acts at the cell surface or needs to be ingested by the cell.

Materials and methods. Enzyme-digested toxin labelled with L-leucine-H^3 was prepared as previously described[8]. The digest containing the activated toxin in 0.05 M cyclohexylaminopropane sulfonic acid (Caps) buffer pH 10.5 was passed through a 2.5×25 cm column of Sephadex G-50 medium equilibrated in the same buffer. Excluded peptides were pooled and refrigerated until required.

1 g Sephadex G-25 fine beads were swollen and activated with cyanogen bromide as described for agarose beads by Cuatrecasas and Anfinsen[9]. The activated beads (~4 ml) were washed with 200 ml Caps buffer pH 10.5 and then added to the pooled excluded peptides from the G-50 column and gently rocked overnight at 23 °C. Unbound peptide was removed by filtration and the beads washed with 200 ml Caps buffer followed by 50 ml of buffered insect saline (BIS)[8].

Assays were conducted by adding 0.1 ml of test material in BIS to 0.1 ml of BIS containing 1.5–2.0×10^5 Cf-124 cells[10] and incubating for 30 min at 28 °C. The viable cells remaining at the end of the period were detected by measuring the residual ATP. The ATPases normally released by dead cells would rapidly hydrolyze any ATP free in solution. They were destroyed, and the incubation terminated, by adding 2 ml boiling 0.05 M Tris buffer pH 7.7 and boiling for an additional 10 min. ATP was determined by the luciferin-luciferase reaction[11]. Each experiment included untreated controls and maximum response controls determined with unbound toxin. The percentage reduction in ATP compared to the untreated control was used as an estimate of the percentage reduction

The reduction in cellular ATP of Cf-124 cells resulting from exposure to activated δ-endotoxin of *Bacillus thuringiensis* covalently bound to Sephadex G-25 fine beads

	Δ ATP %	p
Bound toxin + antibody	− 8	< 0.001
Heated bound toxin	− 23	< 0.001
Bead-free supernatant	− 16	< 0.001
Bound toxin	− 50	
Free toxin	− 50	

p is the probability that the value is not different from that of bound toxin (ANOVA).

in viable cells. Each treatment was replicated 6 times and the ATP of 3 aliquots of each replicate was measured. Treatments were compared using analysis of variance.

Antisera were a gift of Dr J. Krywienczyk of this Institute. Dosages, toxin concentrations and amounts of toxin bound were measured by scintillation counting of the radioactive toxin.

Results and discussion. 1 g dry G-25 Sephadex beads bound $0.76\text{–}1.2 \times 10^7$ dpm of excluded peptide corresponding roughly to 10 mg of toxin bound/g of beads. The binding reaction is very fast and the overnight rocking at pH 10 served to inactivate any unoccupied binding sites[9]. Since these peptides were excluded from Sephadex G-50 they must be bound to the external surface of the G-25 bead and, therefore, are available to bind to cells.

While the level of statistical significance of observed differences varied from one experiment to the next, bound toxin consistently caused a greater reduction in ATP than the other treatments and the relative effects of the various treatments were as depicted in the table.

The column labelled Δ ATP % represents the percent reduction in ATP due to the treatment. In this experiment treatment with bound toxin resulted in a 50% reduction in ATP, the maximum response obtainable with high doses of unbound toxin. The amount of bound toxin was the same in each treatment. The 2nd column represents the probability that a particular result is not different from the result obtained with bound toxin.

The primary control was bound toxin treated with sufficient toxin-specific rabbit antibody to completely inactivate the toxin. This treatment also acts as a nonspecific protein control indicating that nontoxin protein will not produce the effect. Boiling for 1 h reduced the activity of the bound toxin only by 50% but the difference between heated and unheated toxin was highly significant. This treatment and the antibody control also indicate that the beads are not responsible for the effect observed in the bound toxin treatment.

Finally, to determine if the effect observed was due to toxin that was resolubilized during the incubation period, bound toxin was incubated for 30 min in the absence of cells and the bead-free incubate assayed against cells. Some reduction in ATP is observed but not sufficient to account for the bound toxin results.

Thus, bound toxin causes a reduction in the ATP levels of Cf-124 cells that is prevented or reduced by antitoxin antibody or heat denaturation but is not due to the presence of nontoxin protein, the beads themselves, or to unbound toxin. Since the beads are very much larger than the cells the evidence strongly supports the conclusion that toxin acts at the cell surface and does not need to be internalized to cause cell death.

These results do not necessarily conflict with recently published evidence that δ-endotoxin can uncouple oxidative phosphorylation in mitochondria isolated from midguts of a susceptible lepidopteran[12]. Similar effects in whole cells were not demonstrated. Nevertheless, uncoupler agents are electrogenic ionophores and can act ionophorically on lipid vesicles as well as mitochondria[13,14]. In mitochondria they abolish all transmembrane ion gradients[13] and likely have the same effect on plasma membranes. Abolition of transmembrane ion gradients is compatible with the effects of δ-endotoxin as presently understood.

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Irreversible depigmentation of dark mouse hair by T-2 toxin (a metabolite of *Fusarium sporotrichioides*) and by calcium pantothenate

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Summary. T-2 toxin, a trichothecene metabolite of several Fusarium spp. causes depigmentation of dark mouse hair at the site of its application. Calcium pantothenate, though usually considered as antigreying factor, caused depigmentation at the site of its i.p. injections, at high concentration.

Depigmentation of dark hair can be caused by a variety of chemical and physical agents[1]. Carcinogenic alkylating agents are particularly effective in this respect, as shown in the case of N-methyl-N-nitrosourethane; their action has been interpreted as due to the depletion of intracellular thiols by alkylation[2].