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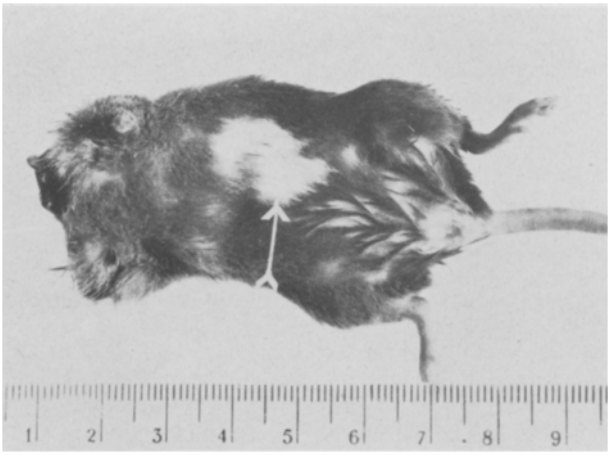
Summary. T-2 toxin, a trichothecene metabolite of several Fusarium spp. causes depigmentation of dark mouse hair at the site of its application. Calcium pantothenate, though usually considered as antigreying factor, caused depigmentation at the site of its i.p. injections, at high concentration.

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].



Depigmented area (arrow) in a C57BL female mouse killed 23 months after the first and 21½ months after the last of 5 doses (0.1–0.2 ml) of calcium pantothenate (80–100 mg/ml) injected i.p. into this area.

In recent experiments, T-2 toxin (3α-hydroxy-4β, 15-diacetoxy-8α-(3-methylbutyryloxy)-12,13-epoxy-Δ^9-trichothecene) a secondary metabolite of *Fusarium sporotrichioides* and of certain other species of Fusarium, proved to resemble N-methyl-N-nitrosourethane in being carcinogenic for the digestive tract in rats[3]; and also in inducing depigmentation when applied to the skin of dark mice. Solutions of T-2 toxin, containing 0.2–0.3 mg/ml in 10% aqueous ethanol, when applied to the clipped intrascapular region of C57BL mice (received from the MRC Animal Laboratory Centre, Carshalton, Surrey) caused local irritation, hyperaemia, oedema, ulceration and scab formation; when the scab fell off the healed area remained depilated and in some animals was surrounded by areas of depigmented hair. The necrotising effect of T-2 toxin is caused evidently by higher concentrations than the depigmentation, this may be due to the higher sensitivity of the biochemical reactions involved in the formation of melanins, than those responsible for cell survival.

It has usually been considered that pantothenic acid is an antigreying factor. However, when some of the T-2 toxin-treated mice were concurrently given aqueous solutions of calcium pantothenate, 80–100 mg/ml, by i.p. injections (0.1–0.2 ml/mouse), this did not prevent the local effects of T-2 toxin; moreover at the site of the i.p. injections of calcium pantothenate ulceration sometimes occurred, and after healing the hair in this area became permanently depigmented (as shown in the figure). Similar depigmentation of the hair was seen in mice which were given only calcium pantothenate, by i.p. injections, and no T-2 toxin.

Calcium pantothenate might possibly interract with thiol compounds (and form CoA?); depletion of the intracellular thiols may also be the result of treatment with T-2 toxin, which contains an epoxide ring in its structure. However, further experiments are needed in order to evaluate quantitatively the relation of the agents causing depigmentation, to the various intracellular thiol species, the role of CoA and of other biochemical parameters, which may be involved in the process of melanin formation.

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Effect of food and light schedule on bile flow in the rat

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Summary. In the rat after food intake, whether during the dark or light period, bile flow increases. Food intake seems to be a major factor in the circadian rhythm of bile secretion.

Circadian rhythms have been described in rats for body weight, liver weight, hepatic cell regeneration[1], hepatic glycogen and glycogenolysis, various hepatic enzyme activities as well as enzyme induction[2–4]. Bile flow, biliary concentrations and excretory rates of bile salt, cholesterol and phospholipid also follow a circadian rhythm with a peak at midnight and nadir at noon for bile salts[5]. The bile salt non-dependent fraction of hepatic bile is maximal during the night and early morning and minimal at the end of the light period[6]. Rats eat during the night and sleep during day time. The aim of the present work was to study whether food or darkness were the main stimuli in bile flow increment.

Material and methods. 36 male Wistar rats were housed for 10 days before study in a dark room at constant room temperature and humidity. Rats were lighted with artificial light between 08.00 h and 20.00 h and fed a standard diet from 20.00 h to 08.00 h in group 1 (18 rats), and from 08.00 h to 20.00 h in group 2 (18 rats). Water was given ad libitum in both groups. In each group, experiments were performed either at 08.00 h (9 rats) or 17.00 h (9 rats). The animals were anesthetized with pentobarbital (Nembutal Abbott). Body temperature was maintained between 37.5 and 38.5 °C on heating tables. Renal pedicles were ligated and 5 μCi of ^{14}C erythritol (Amersham) was injected (0.1 ml) via the penis vein. 1 h later, the carotid artery and the bile duct were canulated. Basal bile flow was measured during the first 30 min following bile duct canulation. Arterial blood was sampled (100 μl) at each mid 10 min bile flow period. At the end of the experiment, liver and stomach were weighed. Radioactivity was measured on bile and plasma by liquid scintillation spectrometry (Nuclear Chicago). Quenching was determined by automatic external standardization. The comparison of means was performed with the Student t-test.

Results. Results concerning bile flow (Bf), b.wt, stomach weight (s.wt), liver weight (l.wt) and bile over plasma erythritol ratio (B/P) are given in the table. In group 1 at the end of the feeding period, that is to say in the morning, s.wt, l.wt and Bf whether expressed per 100 g b.wt^{-1} or per g l.wt^{-1} are significantly higher than at the end of the fasting period. In group 2 these values are significantly higher at the end of the feeding period, that is to say at 17.00 h. However Bf when expressed per g l.wt^{-1} is not significantly different. This is due to a very high increase in l.wt and can be appreciated by a significant increase in the l.wt/b.wt ratio. Erythritol B/P ratios are not significantly different in each group.