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Bacterial Toxins: a Table of Lethal Amounts

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SUMMARY

The amounts of bacterial toxins and of some plant and animal proteins that kill humans, monkeys, mice, guinea pigs, and rabbits are tabulated and are discussed in the light of guidelines for the cloning of genes coding for toxins.

SELECTION OF DATA AND SOURCES OF ERROR

The values in Table 1 have been recalculated from the original data to minimize copying errors in reviews, but considerable sources of error remain.

Almost always the major problem in interpreting the literature is to establish the purity of the preparations used and the degree of inactivation suffered during isolation. An attempt was made to list toxicity values obtained only from homogeneous material; except for the toxins that are so abundant in filtrates that purification is simple, this principle entailed the omission of many values obtained before modern methods of protein purification and analysis were available.

A few values (for the anthrax toxin complex, *Clostridium perfringens* beta-toxin, and *Yersinia pestis* murine toxin) are included even though the published data do not allow purities of the preparations to be estimated. These values have been placed in brackets and "<" has been used to emphasize that the figures are maximum values. Some even more suspect data have been relegated to footnotes, and a few toxins have been listed which are probably lethal but for which no data have been found. In general, although the literature frequently contains widely different estimates of toxicities, only the most lethal values are included in the table because these probably represent the purest material and the least inactivation. For toxins that require partial proteolysis for full expression of activity, values are listed only after activation.

The opposite problem of spuriously high potencies arises for proteins of limited toxicities that were isolated from the products of bacteria which produce several toxins. Staphylococci, streptococci, and clostridia are examples. Many products prepared from *C. perfringens*, including commercial enzymes, are contaminated with the cytolytic theta-toxin (63).

A third source of error is the inherent inaccuracy of the determinations themselves. The number of animals used to determine toxicities is frequently insufficient for the accuracy claimed, but the offense is usually only a statistical one, for great accuracy is of no merit in these determinations. The amount of toxin required to kill a particular animal is specific for that animal on that occasion and clearly cannot be measured with any precision. It can only be estimated from other individual animals whose physiological conditions may not be the same. A particularly severe variation concerns the pyrogenic toxins, the apparent lethalities of which vary over several orders of magnitude according to the endotoxin load of the test animals.

Workers have used a variety of routes of injection for toxins being tested. When specified, the route is listed in the table. Intravenous injection is often a few fold more effective than intraperitoneal injection. Intramuscular or subcutaneous injections are often severalfold less effective than intravenous ones. Intracranial or intraspinal values are not given, although these routes are much more effective for many toxins, both the classical neurotoxins (10) and others.

TABLE 1. Lethal amounts of bacterial toxins

Toxin type		Lethal quantity per kg of body wt[a]				
Organism	Toxin name	Mice	Guinea pigs	Rabbits	Monkeys	Humans
I. Bacterial proteins						
Aeromonas hydrophila	Aerolysin	(7 μg i.v.)[12]				
Bacillus anthracis[b]	Lethal factor (with protective antigen)	[<114 μg i.v.[32]: rat]				
	Factor I (with factors II & III)	[<200 μg[80]]				
Bacillus cereus	Cereolysin[c]	40–80 μg[13]				
Bacillus cereus	Enterotoxin (causes vomiting)	(15 mg i.v.)[36]				
Bacillus spp.	Oxygen-labile hemolysins[c]					
Bordetella pertussis	Heat-labile toxin, pertussigen	15 μg[68], 21 μg[6] i.p.				
Clostridium bifermentans and other *Clostridium* spp.	Lecithinase[d]					
Clostridium botulinum						
Type A	Neurotoxin	(1.2 ng i.p.)[50]	(0.6 ng)[e]	(0.5 ng)[e]	(0.5–0.7 ng)[e]	(ca. 1 ng)[e]
Type B	Neurotoxin (proteolytically activated)	(0.5 ng i.p.)[44] 1.2 ng i.p.,[27] 2 ng i.p.[49]	0.6 ng i.p.[49]			
Type C1	Neurotoxin (proteolytically activated)	1.1 ng i.v.[86]	(ca. 1.1 ng)[e]	(ca. 0.15 ng)[e]	(ca. 0.4 ng)[e]	
Type C2	Neurotoxin (proteolytically activated)	1.2 ng i.p.[65]				
Type D	Neurotoxin	0.4 ng i.p.[23]	0.1 ng[e]	0.08 ng[e]	40 ng[e]	
Type E	Neurotoxin	(1.1 ng)[35]	0.6 ng[e]	1.1 ng[e]	1.1 ng[e]	
Type F	Neurotoxin (proteolytically activated)	2.5 ng i.v.[66]				
Clostridium difficile	Enterotoxin, toxin A	500 ng i.p.[88]				
	Cytotoxin	220 μg i.p.[88]				
Clostridium perfringens						
Type A	Alpha-toxin, lecithinase	3 μg i.v.,[75] 5 μg[15]				
Type A	Kappa-toxin	1.5 mg i.v.[40]				
Type A	Theta-toxin, perfringolysin O[c]	13–16 μg i.v.[81]		5–8 μg i.v.[81]		
Type A	Enterotoxin	(140 μg i.v.)[83] 81 μg i.v.[64]				
Types B & C	Beta-toxin	[<400 ng[94]][f]				
Types B & C	Delta-toxin	(5 μg i.v.)[4]				
Types B & D	Epsilon-toxin (activated by trypsin)	(100 ng)[89]				

TABLE 1—*Continued*

Toxin type		Lethal quantity per kg of body wt[a]				
Organism	Toxin name	Mice	Guinea pigs	Rabbits	Monkeys	Humans
Clostridium tetani	Tetanus toxin, tetanospasmin[g]	(1 ng)[38]	(ca. 0.3 ng)[g]	(0.05–5 ng)[g]		(<2.5 ng)[g]
Clostridium spp.	Oxygen-labile hemolysins[c]					
Corynebacterium diphtheriae (and certain other corynbacterial spp.)	Diphtheria toxin	(1.6 mg s.c)[10]	(160 ng s.c.)[70,96h]			(≤100 ng i.m.)[8]
Corynebacterium ulcerans	Cytotoxin (sphingomyelinase?)		(120 μg s.c.)[1]			
Escherichia coli	Heat-labile enterotoxins (LT)	250 μg i.v.[i]				
	Heat-stable enterotoxins (ST)[j]					
Legionella pneumophila	Toxin[j]					
Listeria monocytogenes	Listeriolysin[c]	(3–12 μg)[82]				
Proteus mirabilis	Neurotoxins[k]					
Pseudomonas aeruginosa	Toxin A	3 μg i.v.[22,87]				
	Protease(s)	4 mg i.v.[54]				
Shigella dysenteriae	Neurotoxin[l]	1.3 μg i.p.[92] 450 ng i.v.[67]	>9 μg i.v.[m]	<0–9 ng i.p.[92]	ca. 1 ng i.v.[20m]	
Staphylococcus aureus	Alpha-toxin, alpha-lysin	40–60 ng i.v.[14,55]		1.3 μg[55]		
	Beta-lysin[n]					
	Gamma-lysin[o]					
	Delta-lysin	(110 mg i.v.)[45]	(30 mg i.v.)[45]	ca. 40 mg[93]		
Staphylococcus aureus	Enterotoxin A[p]					
	Enterotoxin B[p]				20 μg i.v.,[26]	
	Enterotoxin C				<50 μg i.v.[37]	
	Leucocidin[j]					
	Pyrogenic toxins A, B, C[q]					
Streptococcus pneumoniae	Pneumolysin[c]			1.5 μg i.v.[79]		
Streptococcus pyogenes	Pyrogenic toxins, erythrogenic toxins[q]	3–6 mg i.v.[43]		3.5 mg i.v.[43]		
	Streptolysin O[c]	8 μg i.v.[3]	24 μg i.v.[5]	1–2 μg i.v.[3]		
	Streptolysin S	ca. 25 μg i.v.[r]				
Vibrio cholerae	Cholera toxin, choleragen	250 μg[30i]				
Yersinia enterocolitica	Heat-stable enterotoxin (ST)[j]					
Yersinia pestis	Murine toxin	[<10 μg i.v.][2] [<35 μg,[2] ca. 50 μg[62] i.p.]				

II. Plant proteins[g]					
Adenia digitata	Modeccin	1–10 μg i.p. (rat)[7]			
Abrus precatorius, seeds[f]	Abrin	(700 ng i.v.)[33]	(400–500 ng i.v.)[33]	(30–60 ng i.v.)[33]	>300 ng[h]
Ricinus communis, seeds[f]	Ricin	(2.7 μg i.v.)[33]	<1.1 μg[29]		>500 ng[h]
III. Animal proteins (a selection)					
Presynaptic neurotoxins					
Oxyuranus scutellatus	Taipoxin	(2 μg i.v.)[28]			
Bungarus multicinctus	Beta-bungarotoxin (phospholipase)	14 μg i.p., 40 μg s.c.[51]			
Crotalus	Crotoxin (phospholipase)	82 μg i.v.[21]			
Notechia scutatus	Notexin (phospholipase)	(25 μg i.v.)[28]			
Postsynaptic neurotoxins					
Dendroaspis viridis	Neurotoxin	45–80 μg i.p.[9,78]			
Naja haje	Neurotoxin	50 μg s.c.[61]			
Bungarus caeruleus	Caeruleotoxin	53 μg[18]			
Scorpion	Various neurotoxins	9–144 μg s.c.[60]			
Cnidaria	Various nematocyst toxins	33–70 μg i.v.[16]			

[a] Values are LD_{50}s, except for those in parentheses, which are MLDs. Brackets indicate impure material. Superscript numbers are references. i.v., Intravenously; i.p., intraperitoneally; s.c., subcutaneously; i.m., intramuscularly; p.o., by mouth.

[b] Both groups reported synergistic effects between fractions of the anthrax toxin complex. All material used was of uncertain purity.

[c] Since oxygen-labile hemolysins tend to have similar toxicities, the related toxins produced by other species of *Bacillus* and *Clostridium* may also have toxicities in the range of 10 to 100 μg/kg for mice. The following candidates are described in reference 82: *Bacillus alvei*, alveolysin; *B. laterosporus*, laterosporolysin; *B. thuringiensis*, thuringiolysin; *Clostridium bifermentans*, lysin; *C. botulinum*, lysin; *C. caproicum*, lysin; *C. chauvoei*, delta-toxin; *C. histolyticum*, epsilon-toxin; *C. oedematiens*, delta-toxin; *C. septicum*, delta-toxin; *C. sordellii*, lysin; *C. tetani*, tetanolysin.

[d] Lethal to mice (59).

[e] Where only ratios are given or only crude toxin was used, the values given are calculated from the ratios to mouse toxicities. Data were obtained for type A—monkeys (84), guinea pigs, and rabbits (57)—and types C_1, D, and E (71). Humans are said to be at least as sensitive as mice (58). The toxicities of the botulinum toxins for some other species are tabulated in references 71 (for types C_1, D, and E) and 95 (types A–E). Botulinum toxin is many orders of magnitude less toxic when given orally (47, 58, 67, 84). However, the "progenitor toxins," which appear to be complexes of the toxins with some other material, are more toxic than the toxins themselves when administered by mouth or to the gut (65), presumably because the extraneous material reduces inactivation.

[f] Other reports suggest much lower toxicity for clostridial beta-toxin (e.g., 100 μg/kg [74]), but the value listed is consistent with the finding of a culture filtrate containing 100,000 MLD/ml (69).

[g] References 31, 34, 48, and 95 give lethalities of tetanus toxin administered to the gut and to the brain. Such data are summarized by van Heyningen and Mellanby (91), who also discuss the factors that affect the toxicity of tetanus toxin. The values for other animals are calculated from the ratios to mouse toxicity. The figure for humans is from reference 17. Data for guinea pigs and rabbits are from Wright (95, p. 658), who cites several authors as saying that guinea pigs are about four times as sensitive as mice. The data for rabbits vary considerably.

[h] Assumed 2.5 μg/Lf. One "MLD" of diphtheria toxin is the amount that, injected s.c., kills a 250-g guinea pig in 4 to 5 days. Less toxin is required if this period is extended. There are many confirmations of the 160-ng value.

[i] LT is here assumed to be as lethal as cholera toxin since it is structurally similar and is as potent as cholera toxin in several nonlethal assays (25). Smaller amounts of LT or of cholera toxin are lethal if administered enterally.

[j] No reliable data found.
[k] Crude *Proteus mirabilis* toxin was lethal to mice at 3 mg/kg i.p. (39).
[l] Neurotoxin is strictly a misnomer, for the flaccid paralysis of rabbits caused by shigella toxin is thought to be due to damage to the endothelium of spinal cord blood vessels that causes neurological symptoms secondarily. It appears that the same material is enterotoxic (causing accumulation of fluid in rabbit ileal loops) and cytotoxic (killing epithelial cells in culture by inhibiting their protein synthesis) (42).
[m] The value for monkeys was estimated from the statement that the fatal dose for monkeys (weight unstated) was approximately the same as that for mice (20). The value for guinea pigs was calculated from the ratio of rabbit LD_{50} to guinea pig LD_{50} (24).
[n] An apparent toxicity of *S. aureus* beta-lysin (93) now seems to be attributable to contaminating alpha-toxin. Pure beta-lysin is not lethal to mice at 7 mg/kg i.v. (56).
[o] Partially pure *S. aureus* gamma-lysin was "lethal for mice and rabbits in less than one milligram" (93).
[p] Enterotoxins A and B are not lethal to mice at 2.5 mg/kg but have been reported to enhance the ability of endotoxin to cause lethal shock (85). The following are emetic doses, per kilogram: type A—monkeys, 2 μg p.o. (11), 0.1 μg i.v. (11); humans, 20 ng p.o. (11). Type B—monkeys, 9 μg p.o. (76) or 2 μg p.o. (11), 0.1 μg i.v. (11); humans, 500 ng. p.o. (11). Type C_2—monkeys, 40 ng i.v. (72).
[q] Pyrogenic toxins are of low lethality per se but markedly reduce the amount of endotoxin required for lethal shock (streptococci [43, 76]; staphylococci [77]).
[r] Calculated from the ratio 300,000 hemolytic units/kg mouse LD_{50} (73) to the hemolytic potential of pure streptolysin S: 12×10^6 hemolytic units/mg (46).
[s] The "A chain" toxins, such as robin, curein, hurin, cretin, and alpha-sarcin, are less toxic, with mouse LD_{50}s of >1 mg/kg.
[t] Isotoxins of abrin (52) and ricin (53) exist, which differ in carbohydrate content but not in toxicity.
[u] From evaluations of abrin and ricin as possible cancerostatic agents, it is clear that at least 0.3 μg of abrin or 0.5 μg of ricin per kg is tolerated by humans without serious symptoms (Ø. Fodstad, personal communication).

Values are expressed per kilogram of body weight, assuming, when necessary that the mice weighed 20 g, the guinea pigs weighed 250 g, and the rabbits weighted 3 kg. Such normalization implies a linearity between dose and weight that probably holds only rarely. The assumption has been explicitly challenged for botulinum toxin by Lamanna (47).

For all of these reasons, the values in the table must be interpreted carefully. At best they are accurate to one significant figure. Usually they are provisional values that may be revised downwards and serve now to define only the likely maximum size of the lethal dose.

THE TABLE

Most values are given as 50% lethal dose (LD_{50}) per kilogram. Those in parentheses are minimum lethal dose (MLD), or LD_{100}, per kilogram. Some authors assume that the MLD is about twice the LD_{50}, but there is no constant rule. For botulinum toxin, which was titrated with care, the factor is about 1.6 (47). For tetanus toxin it is about 1.4 (91). For abrin and ricin it is close to 1.0 (33). For the greatest accuracy the time within which the animals die should be specified, but this information is often omitted. The exception is the "MLD" of diphtheria toxin, which has a somewhat different meaning that includes a time of death (see footnote *h*). Values are given as mass of protein, assuming, when necessary, that the protein contained 16% N.

In part I the bacterial proteins are arranged in alphabetical order of parental bacterium. For comparison, the lethalities of some nonbacterial toxins are included. Part II lists plant protein toxins that have been purified and assayed. Part III is not comprehensive but presents a sample of the neurotoxic proteins from snake and invertebrate venoms. More are listed by Tu (90), but most venoms have been assayed only as mixtures and the potencies of the individual components are unknown. The purified venom neurotoxins are nearly always lethal to mice at 10 to 100 μg/kg. Taipoxin is unusually potent.

DISCUSSION

Relevance to Possible Cloning of Genes Coding for Toxins

One reason for compiling these data arose when the National Institutes of Health Recombinant DNA Advisory Committee and its ad hoc working group on toxins considered the dangers that might develop from the cloning of genes for bacterial and other toxins. The discussions resulted in guidelines for cloning toxin genes in *Escherichia coli* (Fed. Regist. **46**:34487, 1 July 1981). A novel feature is a recommendation that

different containment levels should be used for toxins of different lethalities.

During our discussions it became apparent that, whereas the risk to humans would depend on a toxin's toxicity to humans, human data were not often available and would have to be inferred from values obtained with other animals. Our best recourse would be to extrapolate to humans from measurements on other primates. For this reason, the table includes published data for monkeys. Unfortunately, for many toxins the only indication of likely human toxicity comes from experiments with nonprimate mammals, most often mice, and experience shows that there is often a very poor correspondence between toxicity to humans and that to any one small animal (e.g., diphtheria toxin, shigella "neurotoxin"). Nevertheless, we need some way of predicting human toxicities, and it has been proposed that, unless or until direct measurements on primates are made and solely for the purpose of selecting the appropriate containment level in a cloning experiment, humans be assumed to be as susceptible to a particular toxin as the most susceptible of three small mammals, mice, guinea pigs, and rabbits. As the table reveals, there are few toxins for which adequate small animal data are available, but they would not be hard to collect when necessary.

Recommended Containment Levels for Cloning

The guidelines suggest four classes. The divisions between the classes are, perforce, somewhat arbitrary but represent the working group's best sense of convenience and prudence, given the limited knowledge available. For most toxins extra data will be required to determine the appropriate class.

(i) Proteins with an expected 50% lethal dose for humans of 100 ng/kg or less. In effect, this means 50% lethal for humans, for monkeys, or for the most sensitive of mice, guinea pigs, and rabbits. Cloning is prohibited (without special permission of the National Institutes of Health). This group presently contains the botulinum toxins, tetanus toxin, the shigella neurotoxin, and diphtheria toxin. Others might enter this group when more data become available.

(ii) Proteins with an expected 50% lethal dose for humans of >100 ng/kg and <1 μg/kg. Cloning is permitted under P2 + EK2 or P3 + EK1 containment. Abrin seems likely to belong to this group, as do ricin, modeccin, *C. perfringens* epsilon-toxin, and *C. difficile* enterotoxin.

(iii) Proteins with an expected 50% lethal dose for humans of 1 to 100 μg/kg. Cloning is permitted at P1 + EK1 containment. Extrapolation to humans from the small-animal data places streptolysin O in this class, and it appears likely that other oxygen-labile hemolysins will belong here too, as well as many other toxins, but the data are usually not sufficient to allow decisions yet.

In addition, cloning of cholera toxin-like and ST (heat-stable)-like enterotoxins is permitted under P1 + EK1 containment, even if they should prove to be more potent than 1 μg/kg for humans, for the reasons discussed below.

(iv) Proteins of low toxicity. These proteins, lethal to humans at over 100 μg/kg, are not subject to specific restrictions on cloning (except for the enterotoxins in group 3). An example is the delta-lysin of *Staphylococcus aureus*.

Risks Associated with Cloning Toxin Genes in *Escherichia coli*

These categories and the guidelines apply only to cloning in *E. coli*. The risk inherent in using a different host would depend on the habits of this organism. It must be emphasized that the habits of a gene's former host, including its ability to cause disease or to exchange genetic information, are no longer relevant once the gene is transplanted. For example, the knowledge that *C. tetani* exists in the normal bowel without pathology does not make it any safer to transfer the gene for tetanus toxin into another intestinal organism. For *E. coli* the major risk seems to lie in the production of a toxin in the intestine by either *E. coli* itself or another intestinal organism that acquired the toxin gene from *E. coli*. We can imagine three types of dangerous outcomes.

(i) Some of the toxin might pass out of the bowel into the general circulation and damage distant tissues. This would be most apparent for those such as tetanus and botulinum toxins, which have no effect on the bowel itself but which inactivate neural synapses. That some botulinum toxin escapes into the circulation is implicit in every case of botulism: possible mechanisms have been discussed by Bonventre (19). Only about 1 part in 100,000 of orally administered botulinum toxin escapes (47), but a greater proportion might escape if the toxin were to be made in the gut itself and avoid inactivation by the stomach. Wright (95, p. 420), in reviewing a few experiments in which botulinum toxin was placed in the ileum, ileal loops, and colonic loops, concluded, "What slender evidence there is available thus suggests that most of the absorption of these toxins must take place in the stomach or in the upper portions of the small intestine." Shigella neurotoxin is substantially inactivated in the stomach (20). The risk must be greater for adults in whom passage of proteins from the intestine is rendered more likely by such conditions as ulcers or intestinal rupture or for neonates.

(ii) Many of the toxins that are lethal when injected parenterally are cytotoxic and if pro-

duced in the intestine will presumably cause necrosis and ulceration in the mucosa and consequently diarrhea or dysentery. The cytotoxic enterotoxin of *Shigella dysenteriae* is thought to act thus (41). The mucosal damage might also be followed by a greater leakage of the toxin into the circulation, which would pose an additional risk.

(iii) The noncytotoxic enterotoxins such as cholera toxin and the heat-stable and heat-labile enterotoxins of *E. coli* would presumably cause secretion in the same way that they do in the natural diseases. Despite the fear historically associated with the word cholera, the dehydration consequent on the diarrhea is completely reversed by oral and intravenous administration of electrolyte solutions and, given proper care, the risk to an experimenter from a neocholera organism may be limited to discomfort.

Except for the enterotoxins, there are few data on the safe amounts of toxins in the guts of experimental animals, let alone in humans. We can only proceed on the temporary assumption that a relation exists between enteral and parenteral toxicities. We must assume that a toxin which kills when minute amounts are administered to the blood may also be a significant danger when produced in the gut, and that the more toxic it is, the greater the barriers that should be erected to restrict the toxin's production in the intestine. This may be accomplished either by imposing physical containment or by using strains of *E. coli* that do not colonize and have less opportunity to transfer their genetic information to abundant intestinal residents.

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