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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.

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			I ethal on	I ethal quantity ner kg of body wta	wta	
Toxi	Toxin type		nh minor	Canada San and Carrier		Transac
Organism	Toxin name	Mice	Guinea pigs	Rabbits	Monkeys	Humans
I. Bacterial proteins Aeromonas hydrophila Bacillus anthracis <sup>b</sup>	Aerolysin Lethal factor (with protec-	(7 μg i.v.) <sup>12</sup> [<114 μg i.v. <sup>32</sup> : rat]		·		
	Twe anugen) Factor I (with factors II &	$[<\!200~\mu {\rm g}^{80}]$				
Bacillus cereus Bacillus cereus	Cereolysin <sup>c</sup> Enterotoxin (causes vomit-	40–80 μg <sup>13</sup> (15 mg i.v.) <sup>36</sup>				
Bacillus spp. Bordetella pertussis Clostridium bifermentans and other Clostridium	mg) Oxygen-labile hemolysins <sup>c</sup> Heat-labile toxin, pertussigen Lecithinase <sup>d</sup>	15 µg <sup>68</sup> , 21 µg <sup>6</sup> i.p.				
spp. Clostridium botulinum Type A Type B	Neurotoxin Neurotoxin (proteolytically		(0.6 ng) <sup>e</sup>	.(0.5 ng) <sup>e</sup>	(0.5–0.7 ng) <sup>e</sup>	(ca. 1 ng) <sup>e</sup>
Type C1	activated) Neurotoxin (proteolytically	1.2 ng i.p., 2 ng i.p., 1.1 ng i.v. <sup>86</sup>	0.6 ng 1.p.* (ca. 1.1 ng) <sup>e</sup>	(ca. 0.15 ng) <sup>e</sup>	(ca. 0.4 ng) <sup>e</sup>	
Type C2	activated) Neurotoxin (proteolytically	1.2 ng 1.p. <sup>65</sup>				
Type D Type E Type F	activated) Neurotoxin Neurotoxin Neurotoxin	0.4 ng i.p. <sup>23</sup> (1.1 ng) <sup>35</sup> 2.5 ng i.v. <sup>66</sup>	0.1 ng° 0.6 ng°	0.08 ng° 1.1 ng°	40 ng <sup>e</sup> 1.1 ng <sup>e</sup>	
Clostridium difficile	activated) Enterotoxin, toxin A Cytotoxin	500 ng i.p. <sup>88</sup> 220 µg i.p. <sup>88</sup>				
Clostridium perfringens Type A Type A	Alpha-toxin, lecithinase Kappa-toxin			•		
Type A Type A	Theta-toxin, perfringolysin O <sup>c</sup> Enterotoxin			5-8 µg i.v.°.		
Types B & C Types B & C Types B & D	Beta-toxin Delta-toxin Epsilon-toxin (activated by	1.v7 [<400 ng <sup>94</sup> ] <sup>f</sup> (5 µg i.v.) <sup>4</sup> (100 ng) <sup>89</sup>				

>300 ng" >500 ng"

(30–60 ng i.v.)<sup>33</sup>

(400–500 ng i.v.)<sup>33</sup> <1.1 μg<sup>29</sup>

1-10 µg i.p. (rat)<sup>7</sup> (700 ng i.v.)<sup>33</sup>

(2.7 µg i.v.)<sup>33</sup>

TABLE 1—Continued

Cotestisium         Toxin năme         Mice         Guinea pigs         Rabbits         Mondeys         Huma           Cotorridium tetani         Tetanus toxin. tetanospasur- convenenceirum alphthera toxin corpusaterum alphthera toxin colorerum alphthera toxin colorer	Organism						
Tetanus toxin, tetanospasm- (1 ng)	Organism			Lethal q	uantity per kg of bod	ly wta	
Tetanus toxin, tetanospasm		COMIT HARIE	Mice	Guinea pigs	Rabbits	Monkeys	Humans
Her- Diphtheria toxin         Oxygen-labile hemolysins*         (16 mg s.c.) <sup>10,864</sup> her Diphtheria toxin         (1.5 mg s.c.) <sup>10</sup> (160 ng s.c.) <sup>20,864</sup> nase?)         (1.20 μg s.c.) <sup>1</sup> (1.20 μg s.c.) <sup>1</sup> Heat-labile enterotoxins (GT)         (1.20 μg i.v.'         (1.20 μg s.c.) <sup>1</sup> Heat-labile enterotoxins (GT)         (1.21 μg) <sup>82</sup> (1.22 μg) <sup>82</sup> S Listeriolysins         (3-12 μg) <sup>82</sup> (1.23 μg i.v. <sup>22,87</sup> Neurotoxins (GT)         (1.3 μg i.v. <sup>22,87</sup> (1.3 μg i.v. <sup>22,87</sup> Neurotoxin (Apha-toxin, alpha-toxin, alpha-toxin, alpha-toxin         (1.10 μg i.v.) <sup>45</sup> (30 mg i.v.) <sup>45</sup> (2.3 μg i.v. <sup>26</sup> Bela-lysin (Agamic toxin AP)         Enterotoxin AP         (1.10 mg i.v.) <sup>45</sup> (30 mg i.v.) <sup>45</sup> (20 μg i.v.) <sup>27</sup> Enterotoxin AP         Enterotoxin AP         (1.0 mg i.v.) <sup>45</sup> (30 mg i.v.) <sup>45</sup> (30 μg i.v.) <sup>26</sup> Extreptolysin OF         8 μg i.v. <sup>3</sup> (4 μg i.v.) <sup>3</sup> (4 μg i.v.) <sup>3</sup> (4 μg i.v.) <sup>3</sup> Cholera toxin, coxins (Crobera toxin (CXII)         (4 μg i.v.) <sup>2</sup> (4 μg i.v.) <sup>3</sup> (4 μg i.v.) <sup>3</sup> Electrotoxin (CXII)         (4 μg i.v.) <sup>2</sup> (4 μg i.v.) <sup>3</sup> (4 μg i.v.) <sup>3</sup> (4 μg i.v.) <sup>3</sup> E	Clostridium tetani	Tetanus toxin, tetanospasm-		(ca. 0.3 ng) <sup>g</sup>	(0.05–5 ng) <sup>g</sup>		Similaria de Company
Alpha-toxin (sphingomyelin-ase?)   Alexal-lable enterotoxins (ST)     Heal-table enterotoxins (ST)     Heal-table enterotoxins (ST)     Toxin	Clostridium spp. Corynebacterium diphtheriae (and certain other corynbacterial spn.)	Oxygen-labile hemolysins <sup>c</sup> Diphtheria toxin	(1.6 mg s.c) <sup>10</sup>	(160 ng s.c.) <sup>70,96</sup>			(<2.3 ng)* (≤100 ng i.m.) <sup>8</sup>
### Text-stable enterotoxins (STY)    Variable	Corynebacterium ulcerans Escherichia coli	Cytotoxin (sphingomyelin- ase?) Heat-labile enterotoxins (LT)		(120 µg s.c.) <sup>1</sup>			
State Toxin A Protesse(s)         3 μg i.v.²2.87 4 mg i.v.²4 4 mg i.v.²4 4 mg i.v.²4 4 mg i.v.²5 4 hg i.v.²6 450 ng i.v.³6 450 ng i.v.³6 50 ng i.v.³7 50 ng i.v.³8 ng i.v.³8 50 ng i.v.³8 50 ng i.v.³9 50 ng i.v.³9 ng i.	Legionella pneumophila Listeria monocytogenes Proteus mirabilis	Heat-stable enterotoxins (ST) Toxin <sup>7</sup> Listeriolysin <sup>c</sup> Neurotoxins <sup>4</sup>		,	· .		
Alpha-toxin, alpha-lysin  Beta-lysin"  Gamma-lysin"  Gamma-lysin"  Gamma-lysin  Beta-lysin"  Gamma-lysin  Beta-lysin"  Gamma-lysin  Beta-lysin  Beta-lysin  Gamma-lysin  Enterotoxin AP  Enterotoxin BP  Function Sind  Pyrogenic toxins A, B, C <sup>q</sup> Pyrogenic toxins, erythro-  genic toxins, erythro-  genic toxins  Streptolysin S  Ca. 25 µg i.v. <sup>3</sup> Cholera toxin, choleragen  Heat-stable enterotoxin (ST)  Murine toxin  [<355 µg, 2 ca. 50  µg, 2 light, 3 l	r seudomonas aeruginosa Shigella dysenteriae	Toxin A Protease(s) Neurotoxin'	3 µg i.v. <sup>22,87</sup> 4 mg i.v. <sup>54</sup> 1.3 µg i n <sup>92</sup>				
Gamma-Iysin° Delta-Iysin Enterotoxin AP Enterotoxin BP  Enterotoxin BP  Enterotoxin C Leucocidir/ Pyrogenic toxins A, B, Cq Pyrogenic toxins, erythro- genic toxinsq Streptolysin O Streptolysin S Cholera toxin, choleragen Heat-stable enterotoxin (STy) Murine toxin  Can 25 µg i.v.³  Can 25 µg i.v.³  Can 25 µg i.v.³  Cholera toxin, choleragen  [<35 µg i.v.³  Can 25 µg i.v.³  Can 40 mg³³  1.5 µg i.v.³  3.5 mg i.v.³  Can 25 µg i.v.³	Staphylococcus aureus	Alpha-toxin, alpha-lysin Beta-lysin"	450 ng i.v. <sup>67</sup> 40–60 ng i.v. <sup>14,55</sup>	/y µg 1. v	<0–9 ng i.p. <sup>92</sup>	ca. 1 ng i.v. <sup>20m</sup>	
Enterotoxin C Leucocidir/ Pyrogenic toxins A, B, C <sup>4</sup> Pyrogenic toxins, erythro- Streptolysin O <sup>c</sup> Streptolysin S Cholera toxin, choleragen Hear-stable enterotoxin (STy) Murine toxin  Eurococidir/ Pyrogenic toxins A, B, C <sup>4</sup> 8 µg i.v. <sup>3</sup> 3.5 µg i.v. <sup>79</sup> 3.5 µg i.v. <sup>79</sup> 3.5 µg i.v. <sup>79</sup> 3.5 µg i.v. <sup>79</sup> 1.2 µg i.v. <sup>3</sup> 1.2 µg i.v. <sup>3</sup> 1.2 µg i.v. <sup>3</sup> [<10 µg i.v. <sup>3</sup> [<35 µg, ca. 50 µg <sup>20 i</sup> 1.8 µg i.v. <sup>3</sup> 1.2 µg i	Staphylococcus aureus	Gamma-lysin Delta-lysin Enterotoxin A <sup>p</sup> Enterotoxin B <sup>p</sup>	(110 mg i.v.) <sup>45</sup>	(30 mg i.v.) <sup>45</sup>	ca. 40 mg <sup>93</sup>		
Pyrogenic toxins, erythro- genic toxins <sup>q</sup> Streptolysin O <sup>c</sup> Streptolysin S Cholera toxin, choleragen Heat-stable enterotoxin (STy Murine toxin  Pyrogenic toxins <sup>q</sup> 8 µg i.v. <sup>3</sup> 24 µg i.v. <sup>5</sup> ca. 25 µg i.v. <sup>7</sup> Ca. 25 µg i.v. <sup>7</sup> (<10 µg i.v.] <sup>2</sup> [<35 µg, <sup>2</sup> ca. 50 µg <sup>co</sup> i.p.]	iae	Enterotoxin C Leucocidin' Pyrogenic toxins A, B, C <sup>q</sup> Pneumolysin <sup>c</sup>			. •	20 μg i.v., <sup>26</sup> <50 μg i.v. <sup>37</sup>	
Streptolysin S ca. 25 µg i.v.? 24 µg i.v.?  Streptolysin S ca. 25 µg i.v.?  Cholera toxin, choleragen 250 µg <sup>30</sup> !  Heat-stable enterotoxin (STy  Murine toxin [<10 µg i.v.] <sup>2</sup> [<35 µg, <sup>2</sup> ca. 50  µg <sup>62</sup> i.p.]		Pyrogenic toxins, erythrogenic toxins <sup>q</sup> Strentolvein Oc	3–6 mg i.v. <sup>43</sup>		1.5 µg i.v. <sup>79</sup> 3.5 mg i.v. <sup>43</sup>		
Murine toxin		Streptolysin S Streptolysin S Cholera toxin, choleragen Heat-stable enterotoxin (ST)	8 μg i.v.³ ca. 25 μg i.v.² 250 μg <sup>30</sup> ²	24 μg i.v. <sup>5</sup>	1-2 µg i.v.³		
			[<10 µg i.v.] <sup>2</sup> [<35 µg, <sup>2</sup> ca. 50 µg <sup>62</sup> i.p.]				

II. Plant proteins'

Adenia digitata

Abrus precatorius, seeds'

Ricinus communis, seeds'

Ricin Ricinus communis, seeds'

III. Animal proteins (a selec-

[<35 µg, 2 ca. 50 µg<sup>62</sup> i.p.]

<10 µg i.v.]<sup>2</sup>

са. 25 µg i.v.<sup>r</sup> 250 µg<sup>30 i</sup>

Heat-stable enterotoxin (ST)

Yersinia enterocolitica Yersinia pestis

Murine toxin

Cholera toxin, choleragen

8 µg i.v.³

1.5 µg i.v.79 3.5 mg i.v. 43 1-2 µg i.v.³

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3-6 mg i.v.<sup>43</sup>

Pyrogenic toxins, erythro-

Pneumolysin $^c$ 

Streptococcus pneumoniae Streptococcus pyogenes

Streptolysin Oc genic toxins<sup>q</sup> Streptolysin S

>300 ng"	>500 ng"						
	٠,						
(30–60 ng	(',,')						
(400–500 ng	$< 1.1  \mu g^{29}$						
1–10 µg i.p. (rat) <sup>7</sup> (700 ng i.v.) <sup>33</sup>	(2.7 µg i.v.) <sup>33</sup>		(2 µg i.v.) <sup>28</sup>	S.C. 51	82 μg 1.V (25 μg i.V.) <sup>28</sup>	45–80 µg i.p. <sup>9,78</sup> 50 µg s.c. <sup>61</sup>	53 µg <sup>18</sup> 9–144 µg s.c. <sup>60</sup> 33–70 µg i.v. <sup>16</sup>
Modeccin Abrin	Ricin		Taipoxin Dete brancostovie (abourbo	lipase)	Crotoxin (phospholipase) Notexin (phospholipase)	Neurotoxin Neurotoxin	Caeruleotoxin Various neurotoxins Various nematocyst toxins
II. Plant proteins' Adenia digitata Abrus precatorius, seeds'	Ricinus communis, seeds'	III. Animal proteins (a selection)	Presynaptic neurotoxins Oxyuranus scutellatus	Dangaras manicincias	Crotatus Notechia scutatus	Postsynaptic neurotoxins Dendroaspis viridis Naja haje	Bungarus caeruleus Scorpion Cnidaria

<sup>a</sup> Values are LD<sub>50</sub>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.

<sup>b</sup> Both groups reported synergistic effects between fractions of the anthrax toxin complex. All material used was of uncertain purity.

<sup>c</sup> Since oxygen-labile hemolysins tend to have similar toxicities, the related toxins produced by other species of Bacillus and Clostridium may also have laterosporolysin, B. thuringiensis, thuringiolysin, Clostridium bifermentans, Iysin; C. botulinum, Iysin; C. caproicum, Iysin; C. chauvoei, delta-toxin; C. 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, histolyticum, epsilon-toxin; C. oedematiens, delta-toxin; C. septicum, delta-toxin; C. sordellii, Iysin; C. tetani, tetanolysin.

<sup>d</sup> Lethal to mice (59).

the botulinum toxins for some other species are tabulated in references 71 (for types C1, 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 " 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 C1, D, and E (71). Humans are said to be at least as sensitive as mice (58). The toxicities of inactivation.

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)

ing that guinea pigs are about four times as sensitive as mice. The data for rabbits vary considerably.

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 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 say-

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. this period is extended. There are many confirmations of the 160-ng value.

Crude Proteus mirabilis toxin was lethal to mice at 3 mg/kg i.p. (39) No reliable data found

nal cord blood vessels that causes neurological symptoms secondarily. It appears that the same material is enterotoxic (causing accumulation of fluid in 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 spi rabbit ileal loops) and cytotoxic (killing epithelial cells in culture by inhibiting their protein synthesis) (42)

" 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<sub>50</sub> to guinea pig LD<sub>50</sub> (24).

" 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

Partially pure S. aureus gamma-lysin was "lethal for mice and rabbits in less than one milligram" (93)

lowing are emetic doses, per kilogram: type A-monkeys, Žug 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 4 Pyrogenic foxins are of low lethality per se but markedly reduce the amount of endotoxin required for lethal shock (streptococci [43, 76]; 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 folp.o. (11), 0.1 µg i.v. (11); humans, 500 ng. p.o. (11). Type Cz—monkeys, 40 ng i.v. (72)

Calculated from the ratio 300,000 hemolytic units/kg mouse  $LD_{50}$  (73) to the hemolytic potential of pure streptolysin S:  $12 \times 10^6$  hemolytic units/mg The "A chain" toxins, such as robin, curein, hurin, cretin, and alpha-sarcin, are less toxic, with mouse LD<sub>50</sub>s of >1 mg/kg.

staphylococci [77])

" 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 hu-Isotoxins of abrin (52) and ricin (53) exist, which differ in carbohydrate content but not in toxicity mans 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<sub>50</sub>) per kilogram. Those in parentheses are minimum lethal dose (MLD), or LD<sub>100</sub>, per kilogram. Some authors assume that the MLD is about twice the LD50, 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 conta toxins of differ

During our that, whereas on a toxin's were not often inferred from mals. Our best to humans fro mates. For thi lished data fo many toxins th toxicity comes mate mammal ence shows t correspondend that to any or toxin, shigella need some wa and it has bed direct measure solely for the ate containme humans be as particular toxi small mammal As the table which adequat but they wou necessary.

#### Recommended

The guideling sions between what arbitrary best sense of the limited knd extra data wi appropriate cl

(i) Proteins for humans of means 50% le for the most s rabbits. Cloni permission of This group p toxins, tetanu and diphtheri group when n

(ii) Proteins for humans of is permitted containment. this group, as epsilon-toxin,

(iii) Proteins for humans of ted at P1 + E humans from tolysin O in the Vol. 46, 1982

expressed per kilogram of body ing, when necessary that the mice he guinea pigs weighed 250 g, and eighted 3 kg. Such normalization rity between dose and weight that only rarely. The assumption has a challenged for botulinum toxin 17).

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#### THE TABLE

are given as 50% lethal dose gram. Those in parentheses are l dose (MLD), or  $LD_{100}$ , per authors assume that the MLD is  $\mathbf{E} \mathbf{D}_{50}$ , but there is no constant istum toxin, which was titrated factor is about 1.6 (47). For is about 1.4 (91). For abrin and e to 1.0 (33). For the greatest me within which the animals die fied, but this information is often Reption is the "MLD" of diphhach has a somewhat different clades a time of death (see footare given as mass of protein, necessary, that the protein con-

deterial proteins are arranged in less of parental bacterium. For lethalities of some nonbacterial ded. Part II lists plant protein been purified and assayed. Part energy been some proteins from snake and invertions are listed by Tu (90), but we been assayed only as mixtoneries of the individual comnown. The purified venom neuty always lethal to mice at 10 to exin is unusually potent.

#### DISCUSSION

### sible Cloning of Genes Coding for Toxins

or compiling these data arose all Institutes of Health Recombitory Committee and its ad hoce toxins considered the dangers of from the cloning of genes for er toxins. The discussions retions are cloning toxin genes in (Fed. Regist. 46:34487, 1 July ature 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 animais. 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  $\mu$ g/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

(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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