

phagocytic stain.⁴ Suitable control experiments were conducted which showed that the media in which the tyrothricin was dissolved had no effect in the dilutions considered.

Table I shows the actions of tyrothricin and tyrocidine hydrochloride on leucocytes in the presence and absence of serum. This finding is typical of the results obtained in 20 instances.

Table II shows the effect of tyrothricin and tyrocidine hydrochloride upon the amount of phagocytosis taking place, recorded as the percentage of leucocytes containing pneumococci when 50 cells were observed, and also upon the pneumococci

themselves, expressed by the amount of lysis occurring.

When normal leucocytes in saline suspension are treated with the phagocytic stain employed, the nuclei are stained red and the cytoplasm a purplish blue. It was noted that in some of the lower concentrations of tyrothricin and tyrocidine hydrochloride this staining reaction was reversed, the nuclei appearing blue and the cytoplasm red. The cells exhibiting these peculiar staining reactions were also noted to have ingested more pneumococci than those staining in the normal fashion.

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⁴ Lucke, B., Strumia, M., Mudd, S., McCutcheon, M., and Mudd, E. B. H., *J. Immunol.*, 1933, **24**, 455.

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The Acute Toxicity of Choline Hydrochloride in Mice and Rats.*

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Although a number of isolated observations on the acute toxicity of choline have been recorded, no systematic studies are available. The relatively low toxicity of choline is illustrated by the statement of Mott and Halliburton³ that "We have never succeeded in killing an animal by injection of choline or choline hydrochloride." However, the lethal dose of choline has been estimated for several species as shown in the accompanying text table and the generality has been established that animals either die promptly (within 20 min) from the effects of choline or recover, apparently without permanent injury.

No observations on rats are available.

In the tests reported here, choline hydro-

chloride (Eastman Kodak Co.) was given in aqueous solutions as indicated (Table I). Death, when it occurred, followed promptly

Toxicity of Choline Hydrochloride.

Species	Lethal dose	Route	Reference
Mice	700 mg/kg	Subcutaneous	1
Cats	35 mg/kg	Intravenous	1,2
Rabbits	1 g/kg	Rectally	5
	0.11 g/kg	Intravenously	5
	1 g/kg	Subcutaneously	5
Frogs, large	0.1 g	?	4
Frogs, small	0.05 g	?	4

—for large doses within 2-4 min in some of the animals. The symptoms were those previously described by many investigators; in

¹ Arai, K., *Arch. ges. Physiol.*, 1922, **193**, 359.

² Lohmann, A., *Arch. ges. Physiol.*, 1907, **118**, 215.

³ Mott, F. W., and Halliburton, W. D., *Trans. Roy. Soc. Lond.*, B, 1899, **191**, 211.

⁴ Boehm, R., *Arch. Exp. Path. Pharm.*, 1885, **19**, 87.

⁵ Dreyfus, L., *C. N. S. B.*, 1920, **83**, 481.

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mice, salivation, trembling, jerking, cyanosis, clonic shivering convulsions, respiratory paralysis; in rats, these symptoms with, in addition, a marked bleeding from the eyes which appeared to start from the medial canthus. This hemorrhage was observed in about 60% of the rats dying from the drug.

Mice. One hundred six male and female

TABLE I.
Acute Toxicity Data on Choline Hydrochloride for
Mice and Rats.
A. Mice—Intraperitoneal Injection of 2% Choline
Hydrochloride Solution.

No. Mice	Dose, mg	No. dead	% Mortality
16	5.0	0	0
15	5.6	4	27
15	6.0	7	47
15	6.6	6	40
15	7.0	10	67
15	8.0	13	87
15	10.0	13	87

L.D. 50 (probit kill—5.0) = 6.4 mg per mouse or 320 mg per kg.

B. Rats—Stomach Tube Administration of 67%
Choline Hydrochloride Solution.

No. Rats	Dose, g	No. dead	% Mortality
15	.40	0	0
15	.54	3	20
15	.60	3	20
15	.67	6	40
15	.80	9	60
15	1.00	12	80

L.D. 50 (probit kill—5.0) = 0.73 g per rat or 6.7 g per kg.

albino mice weighing 18-26 g were given intraperitoneally various doses of choline hydrochloride as a 2% solution in water. No correlation could be observed between body weight (in these limits) and mortality. The fur around the eyes seemed wet to a distance of about 1 mm; the eyes appeared to darken. On the high doses, all the mice that died did so in 4 min after injection. The L.D. 50 (Table IA) was found to be 320 mg per kg by the method of Bliss.⁶

Rats. Ninety male albino rats of average weight about 150 g but varying from 120-200 g were given by stomach tube various doses of choline hydrochloride as a 67% solution in water. No effect on mortality was observed by this variation in body weight. On autopsy, the blood vessels of the diaphragm and stomach were engorged. The liver and spleen appeared congested. Stomachs were bleached and distended. The hearts stopped in diastole. The L.D. 50 (Table IB) was found to be 6.7 g per kg.

Summary. The analyses of the data indicate an L.D. 50 for choline hydrochloride of the order of 320 mg per kg for intraperitoneal injection in albino mice, and of 6.7 g per kg for stomach tube administration in albino rats.

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⁶ Bliss, C. L., *Ann. Appl. Biol.*, 1935, **22**, 134.