TABLE I.
The α, β, and Cold Agglutinin Titers of Serums from Two Cases of Blackwater Fever.

	Titer at 37°C for 30 min Agglutinins			Titer at 6°C overnight Agglutinins		
	α	β	anti-O	α	β	anti-O
Serum sample 1						
Untreated serum	8,112	128	0	32,448	1,024	32
Serum treated with Group AB erythrocytes	0	0	0	32	32	32
Serum treated with ascarid polysaccharide	0	0	0	32	32	32
Serum sample 2						
Untreated serum	2,028	64	0	8,112	256	32
Serum treated with Group AB erythrocytes	0	0	0	64	32	32
Serum treated with ascarid polysaccharide	0	0	0	32	32	32

These agglutinins behaved as true cold agglutinins since they agglutinated all erythrocytes regardless of blood group, and their action was reversible by changing the temperature back and forth from 6°C to 37°C. The presence of autoagglutinins in blackwater fever has been previously suggested although not proved experimentally by Dameshek.[3] As explained previously, the source of the agglutinogen may be the malarial parasite.

The possible relationship between the presence of cold agglutinins and the development of blackwater fever should also be taken into consideration. Since exposure to cold is one of the conditions known to accelerate the appearance of blackwater fever symptoms, it seems that such a relationship could profitably be studied.

[3] Dameshek, W., *J. A. M. A.*, 1943, **123**, 77.

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Acute Toxicity of Choline Hydrochloride Administered Intraperitoneally to Rats.*†

HAROLD C. HODGE.

From the Department of Biochemistry and Pharmacology, School of Medicine and Dentistry, The University of Rochester, Rochester, N.Y.

Choline hydrochloride is not highly toxic. Mott and Halliburton[1] emphasize the low order of toxicity when they state that "We have never succeeded in killing an animal by injection of choline or choline hydrochloride." However, lethal doses for several species have been reported[1-9] as of the order of 40-60 mg per kg body weight for intravenous administration and of the order of 200-1000 mg per kg for subcutaneous administration.

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† This paper was presented in part before the Division of Biological Chemistry at the 106th meeting of the American Chemical Society, Pittsburgh, Pennsylvania, September, 1943.

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

[2] Boehm, R., *Arch. Exp. Path. Pharm.*, 1885, **19**, 87.

[3] Asher, L., and Wood, H. C., *Zeit. Biol.*, 1899, **37**, 307.

TABLE I.
Dosage-Mortality Table for Rats Grouped by Dosage Ranges.*
(Intraperitoneal Injection: 518 Rats.)

	Concentration of choline hydrochloride solutions							
	200 mg/ml		100 mg/ml		40 mg/ml		20 mg/ml	
Dosage range mg/100 g body wt	No. rats	% mortality	No. rats	% mortality	No. rats	% mortality	No. rats	% mortality
20-24	15	0						
25-29	44	25						
30-34	31	61	22	32				
35-39	46	72	43	54	8	12		
40-44	15	100	28	79	43	19		
45-49					39	41		
50-54			20	80	11	82	9	67
55-59			5	60			30	43
60-64					15	87	22	64
65-69							6	100
70-74							14	36
75-79							23	71
80-84					15	100		
85-89							14	86
Range of LD_{50}	29-34 mg/100 g body wt		37-38 mg/100 g body wt		41-49 mg/100 g body wt		59-75 mg/100 g body wt	

* The statistical work was performed by Elizabeth Street, her assistance is gratefully acknowledged.

In the first series of tests, 4 solutions of choline hydrochloride (Eastman Kodak Co.) of concentrations 200, 100, 40 and 20 mg per ml, respectively, were given several groups of rats. These rats were of various ages and body weights. The LD_{50}'s calculated by the methods of Bliss[10] were found to be of the order of 35, 41, 54, and 74 mg per 100 g body weight, respectively for the 4 concentrations given above. These data were criticized on several grounds by Professor J. H. Burn during a visit to this laboratory. Following his suggestions the experiment was repeated in such a way that on any testing day, at least 4 groups of rats of the same body weight were used. For each such 4 groups of rats, 1 group received each of the choline hydrochloride solutions as before. An attempt was made on each test to obtain approximately identical percentage kills from doses of each concentration.

A large series of rats were studied and found to show about the same order of dependence of mortality on concentration as had been obtained in the earlier, less well controlled test. The scatter in the data was so serious that LD_{50} values are given only as ranges (bottom, Table I—combined data from both series). It is clear that the greater the concentration of choline hydrochloride in solution, the less of the drug is required to kill the average rat.

The symptoms of acute choline poisoning have been described in detail elsewhere. A few points bear additional discussion. The lethal effect is extremely rapid following high doses—in many groups of 15 rats receiving such doses all the rats which died had done so within 5 minutes of injection. Changes in respiration in these rats were closely followed by trembling, convulsive movements, salivation, hemorrhage around the eyes in about two-thirds of the rats (Fig. 1) dying from the drug, cyanosis, respiratory paralysis, and death. The dark stain around the eyes was

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

5 Vogt, K., *Sitzungsber. Abhandl. naturforsch. Ges.*, Rostock, N.F., 1909, **1**, 109.

6 Hunt, R., and Taveau, R. M., *J. Pharm. Exp. Ther.*, 1909, **1**, 303.

7 Dreyfus, L., *C. R. S. B.*, 1920, **83**, 481.

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

9 Hodge, H. C., and Goldstein, M. R., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 281.

10 Bliss, C. I., *Ann. Appl. Biol.*, 1935, **22**, 134.

FIG. 1.
Hemorrhage around the eyes after large doses of choline hydrochloride. The bleeding seemed to start from the medial canthus. Excessive salivation may be noted on the lower jaw of rat on the left.

identified as blood by Dr. H. R. Brown, Jr. On first examinations, no red cells were found and we were on the point of concluding, as has Barnard recently,[11] that some porphyrin was responsible. However, more careful and prompt examinations showed the presence of rapidly hemolyzing red blood cells in a saline suspension of the exudate. The saline suspension gave a positive guaiac test. In a stained smear, small red blood cells were found and several polymorphonuclear leucocytes identified. This hemorrhage may be attributed to the intense parasympathomimetic action of overwhelmingly large doses of choline.

Rats which survived 20 minutes after injection of any dose invariably lived. This ability of the organism to excrete or to metabolize and detoxify large amounts of choline has been repeatedly noted. No symptoms of poisoning were evident in surviving rats at any time during an observation period of a week following the various tests.

Summary. 1. The amount of choline hydrochloride to kill the average rat (LD_{50}) is greater the more dilute the solution in which it is injected intraperitoneally.

2. Expressed as mg per 100 g body weight, the LD_{50}'s are as follows: 29-34 for a solution containing 200 mg of choline per ml; 37-38 for a solution of 100 mg per ml; 41-49 for a solution of 40 mg per ml; and 59-75 for a solution of 20 mg per ml.

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[11] Barnard, R. D., PROC. SOC. EXP. BIOL. AND MED., 1943, **54**, 254.