previously exposed to small amounts of the antibiotic and render them more susceptible to the host response. The fact that nafcillin, of the penicillins studied, showed the highest index of activity in disorganizing the cell walls of susceptible cells and subsequently rendering them vulnerable to lysis by lysozyme and trypsin, might account for its superior therapeutic activity against experimental infections in mice(8). To what extent, if any, this property of nafcillin contributes to its therapeutic efficacy in clinical situations remains to be clarified.

Summary. Resistance of *Staphylococcus aureus* and other bacteria to lysis by lysozyme and trypsin is abolished in cultures grown in sublethal concentrations of antibiotics. Antibiotics which inhibit synthesis of the mucopeptide polymer of bacterial cell walls differ widely in their ability to influence the lysis of cell suspensions. Such differences are revealed by both the speed of reduction of turbidity and final level of turbidity reduction. Under the influence of nafcillin, cells of *Staphylococcus aureus, Streptococcus faecalis* and *Diplococcus pneumoniae* are readily lysed but *Neisseria catarrhalis, Sarcina lutea* and *Gaffkya tetragena* are resistant, suggesting that bacterial cells differ in resistance or susceptibility to enzymic lysis. Lysis is not conditioned by the *in vitro* antibacterial activity of the antibiotics; nafcillin, which has the same activity against *Staphylococcus aureus* as oxacillin, cloxacillin, cephalothin, novobiocin and vancomycin, gives a greater rate and extent of lysis than the other antibiotics when used in comparable concentrations. Of the antibiotics interfering with protein synthesis, only erythromycin gives a significant lytic response. The results suggest that sublethal levels of antibiotics may mediate a favorable interplay between the host tissue environment and the parasite.

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Dimethyl Sulfoxide (DMSO) as a Solvent in Acute Toxicity Determinations. (30574)

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Reports relating to various pharmacological effects of dimethyl sulfoxide (DMSO) have appeared recently in the literature. A review by Block(1) cites references for both physiological and chemical properties of this compound.

Some of the studies showed DMSO to influence permeability and absorption of drugs. For this reason it was decided to investigate the effects of this compound when used as a solvent for toxicity (LD_{50}) determinations. Some data in this area have already been presented(2,3).

Ten quaternary ammonium salts were selected for test because as a class they are poorly absorbed by the oral route. Further selection was made on the basis of physiological activity: 2 ganglionic blocking agents, 2 muscle relaxants, 2 "antispasmodics," 2 parasympathomimetics and 2 cationic germicides were employed.

Methods. Both male and female albino

TABLE I. Acute Toxicity in Rats.

Compound	Distilled water: No. rats/ No. doses	Distilled water: LD_{50}—95% confidence limits (mg/kg)	Distilled water: Slope function—95% confidence limits	50% Dimethyl sulfoxide: No. rats/ No. doses	50% Dimethyl sulfoxide: LD_{50}—95% confidence limits (mg/kg)	50% Dimethyl sulfoxide: Slope function—95% confidence limits
Pentolinium tartrate	40/4	890 (736 –1077)	1.36 (1.06–1.74)	50/5	438 (371 – 517)*	1.45 (1.18–1.78)
Hexamethonium bitartrate	144/4	2920 (2755 –3095)	1.19 (1.14–1.24)	120/5	2410 (2152 –2699)*	1.32 (1.19–1.47)
Decamethonium iodide	50/5	84.5 (72.8– 98.0)	1.27 (1.12–1.44)	40/4	153 (125 – 187)*	1.49 (1.05–2.12)
Tubocurarine chloride	60/6	36.9 (30.5– 44.6)	1.46 (1.18–1.81)	60/5	27.8 (23.6– 32.8)*	1.31 (1.12–1.53)
Homatropine methylbromide	70/7	1710 (1449 –2018)	1.47 (1.26–1.72)	60/6	1720 (1422 –2081)	1.45 (1.19–1.77)
Atropine methylnitrate	50/5	1630 (1405 –1891)	1.34 (1.16–1.55)	60/6	1720 (1578 –1875)	1.21 (1.11–1.32)
Neostigmine bromide	150/5	75.9 (71.6– 80.5)	1.20 (1.12–1.28)	120/6	50.8 (45.4– 56.9)*	1.38 (1.23–1.55)*
Carbachol	40/4	74.0 (61.2– 89.5)	1.36 (1.06–1.74)	100/4	12.8 (10.1– 16.3)*	2.10 (1.44–3.07)
Cetylpyridinium chloride	70/7	428 (369 – 497)	1.35 (1.22–1.50)	69/7	192 (148 – 250)*	1.56 (1.26–1.93)
Benzethonium chloride	50/5	665 (599 – 738)	1.26 (1.08–1.47)	60/6	368 (317 – 427)*	1.41 (1.22–1.64)

* Ratio significance, P = <.05

TABLE II. Acute Toxicity in Mice.

Compound	Distilled water: No. mice/ No. doses	Distilled water: LD_{50}—95% confidence limits (mg/kg)	Distilled water: Slope function—95% confidence limits	50% Dimethyl sulfoxide: No. mice/ No. doses	50% Dimethyl sulfoxide: LD_{50}—95% confidence limits (mg/kg)	50% Dimethyl sulfoxide: Slope function—95% confidence limits
Pentolinium tartrate	70/7	618 (507 – 754)	1.66 (1.31–2.11)	50/5	545 (454 – 654)	1.33 (1.12–1.58)
Hexamethonium bitartrate	162/6	1970 (1728 –2246)	1.63 (1.36–1.96)	120/6	1670 (1465 –1904)	1.44 (1.25–1.66)
Decamethonium iodide	102/5	119 (108 – 131)	1.25 (1.16–1.35)	92/4	115 (104 – 128)	1.26 (1.14–1.40)
Tubocurarine chloride	70/7	59.5 (46.1– 76.8)	1.60 (1.25–2.05)	60/6	33.2 (25.7– 42.8)*	1.80 (1.21–2.68)
Homatropine methylbromide	140/7	1830 (1488 –2251)	2.28 (1.62–3.21)	120/6	1345 (1170 –1547)*	1.48 (1.26–1.73)*
Atropine methylnitrate	80/4	1320 (1100 –1584)	1.56 (1.20–2.03)	80/4	1420 (1246 –1619)	1.35 (1.14–1.59)
Neostigmine bromide	60/6	7.20 (6.00– 8.64)	1.35 (1.15–1.58)	60/6	6.25 (4.92– 7.94)	1.59 (1.16–2.18)
Carbachol	60/6	42.3 (35.0– 51.2)	1.55 (1.20–2.00)	58/6	17.6 (15.0– 20.6)*	1.44 (1.21–1.71)
Cetylpyridinium chloride	60/6	195 (167 – 228)	1.37 (1.18–1.59)	70/7	108 (89 – 131)*	1.45 (1.22–1.73)
Benzethonium chloride	60/6	485 (404 – 582)	1.44 (1.18–1.76)	50/5	338 (284 – 402)*	1.42 (1.15–1.76)

* Ratio significance, P = <.05

mice (Charles River CD-1 strain) ranging in weight from 14 to 29 g and male albino rats (Charles River CD strain) ranging in weight from 158 to 293 g were used in this study.

Weight ranges were dictated by the supply of animals; over 3,000 mice and rats were used. On any given day of testing, the ranges were much narrower than listed.

All animals were fasted approximately 18 hours before oral dosing. A constant volume of 16 ml/kg was used for both mice and rats; preliminary tests showed this volume of 50% DMSO to be well tolerated.

Two acute oral toxicities, drug in water *vs* drug in 50% DMSO, were made on a single day. When using the DMSO-water mixture, drugs were first dissolved in water and then mixed with DMSO to the desired volume and 50% concentration.

In some instances, as expected with poorly absorbed compounds, 10 animals per dose were insufficient to give a satisfactory mortality curve. In these cases, the test was repeated and the cumulative data utilized.

Statistical evaluation of the data was by the method of Litchfield and Wilcoxon(4) and based on 14-day mortality. Values reported are the LD_{50}, the slope of the mortality curve and their respective 95% confidence limits.

Results. Tables I and II show the results obtained.

A. Ganglionic blocking agents. 1. Pentolinium tartrate—50% DMSO significantly increased toxicity in rats while having no effect in mice. 2. Hexamethonium bitartrate—50% DMSO significantly increased toxicity in rats. No change was noted in the mouse toxicity.

B. Muscle relaxants. 1. Decamethonium iodide—In rats, DMSO decreased toxicity to a significant degree. This was the only instance in which toxicity was decreased. There was no difference in mice. 2. Tubocurarine chloride—A significant increase in toxicity was noted with DMSO in both mice and rats.

C. "Antispasmodics." 1. Homatropine methylbromide—There was no significant change in rat toxicity. The slopes of the mortality curves in mice were significantly different from each other, with the DMSO being considerably steeper. The LD_{50} ratio of the two also showed a significant difference. 2. Atropine methylnitrate—No significant changes were found in either rat or mouse toxicities.

D. Parasympathomimetics. 1. Neostigmine bromide—A significant increase in toxicity with DMSO was found in rats, along with a change in slope; no change was found in mice. 2. Carbachol—The greatest changes in toxicity occurred here. This compound in 50% DMSO was 2.4 times more toxic than when given in water to mice; with rats, toxicity in DMSO was almost 6 times greater than in water.

E. Cationic germicides. 1. Cetylpyridinium chloride—Both rats and mice showed significant increases in toxicity with DMSO. 2. Benzethonium chloride—Here again, DMSO increased toxicity in both rats and mice.

These results show that DMSO, when used as a solvent for quaternary ammonium salts of varied pharmacological effects, can influence oral LD_{50} values in rats and/or mice. The changes ranged from none to statistical significance, which in some cases on a practical basis may be doubtful (A-2, C-1, D-1), up to a highly significant 6-fold increase in toxicity. More changes were seen in rats than in mice. Decreased toxicity with DMSO was obtained in only one instance, decamethonium iodide in rats. Changes of slope were seen in 2 instances, homatropine methylbromide in mice and neostigmine bromide in rats. Only the former is of practical importance.

This study emphasizes something beyond its original purpose. It points up the fact that caution must be exercised when a vehicle, other than water, is selected as the carrier for toxicity testing of drugs. This is by no means a new or original thought but it is brought to the fore by these results.

Summary. Dimethyl sulfoxide and water were compared as solvents for determination of LD_{50} values by the oral route in rats and mice using 10 quaternary ammonium salts as test compounds. Toxicity changes were obtained in some instances with 50% DMSO; more changes were observed in rats than in mice and the two species did not always parallel each other. When toxicity was altered by DMSO, it increased in all instances

except one. Slope was changed in only 2 tests, one each in rats and mice but not with the same compound.

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In vitro Culture of IRC 741 Rat Leukemia.* (30575)

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IRC 741 rat leukemia, also known as Dunning leukemia, is an acute leukemia that occurred spontaneously in a Fischer line 344 female rat bearing the transplanted acetylaminofluorene-induced mammary adenocarcinoma IRC 741(1), and has since been carried *in vivo* in various laboratories. The pathophysiological features of the leukemia have been described(2), and several reports on its use in screening chemotherapeutic agents have appeared(3-8).

We felt that it would be of interest to perform biochemical, karyogenetic, and chemotherapeutic studies with parallel cultures of IRC 741 leukemia *in vivo* and *in vitro*. To our knowledge, no previous reports have been made of *in vitro* culture of this leukemia, hence we would like to summarize our studies of its growth *in vitro*.

Materials and methods. We obtained IRC 741 leukemia from Dr. Dunning in November 1963 in the form of solid tumor transplants growing in Fischer rats. A suspension of single cells was prepared by mincing solid tumor masses, suspending the particles in Ringer's solution, and screening the fluid. This suspension was used to initiate an ascites form of the tumor. Both the solid and ascites forms of the leukemia have since been carried in our laboratory by weekly transfer in Fischer rats.

In December 1963, a biopsy of a solid tumor was minced, and was treated with 0.5% collagenase for 1 hour at 37°C in a rotary shaker. The resulting single-cell suspension was inoculated into various cell-culture media under a single layer of perforated cellophane in T-15 flasks. Immediate proliferation occurred in a culture grown on medium RPMI #906(9) supplemented with 5% fetal calf serum. The cells did not adhere firmly to the glass, and hence subcultures of the cells in the supernatant fluid were established in spinner cultures.

Almost all subsequent studies have utilized suspension cultures. A detailed description of the methods used has been published elsewhere(9). Cell counts and viability determinations were made by direct observation of cells exposed to 0.1% trypan blue and placed in Speirs-Levi counting chambers. Cell counts and glucose levels were determined daily. When necessary, adjustment of the pH to 7.2-7.4 was done daily with $NaHCO_3$ or NaOH. Preparations for chromosome studies were made according to methods that we have previously used for cultured cells(10). Cells grown both *in vivo* and *in vitro* have been stored in liquid nitrogen for extended periods, with good viability after recovery. They are usually frozen in cell-culture medium supplemented with 20% fetal calf serum and con-

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