Figure 4—*LD$_{50}$ of N-myristyl-β-aminopropionate in mice.*

finding that a single short-term exposure of the peritoneal membrane to N-myristyl-β-aminopropionate solution gave an accelerated dialysis effect which lasted throughout at least five subsequent exchanges with standard dialysis fluid. This would make possible the effective use of this accelerator with minimum exposure to the patient. The absorption tests also demonstrated that, although N-myristyl-β-aminopropionate is rapidly taken up from the dialysis fluid, the resulting plasma levels are quite low and appear to be well below concentrations that would result in acute toxicity. With its comparatively short 5-hr. biological half-life (as calculated from the terminal phase of the blood curves), practically all surfactant absorbed into the circulation should be eliminated from the blood in less than 48 hr. These findings should enhance the potential for the clinical use of N-myristyl-β-aminopropionate as an accelerator during peritoneal dialysis. Further toxicological studies are essential, of course, before this substance might be tested in man.

The mechanism of action of the surfactant is not yet known. Studies to determine the mechanism are being conducted but are not yet conclusive.

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Survival of *Staphylococcus aureus* on Pharmaceutical Oral Solid Dosage Forms

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Abstract □ The relationship of temperature, relative humidity, size of inoculum, and duration of storage on survival of *Staphylococcus aureus* inoculated onto surfaces of 17 commercial tablets and one gelatin capsule was determined. The data were analyzed using a two-way factorial analysis of variance. Decreased survival was associated with an increase in each of the variables, within limits of the experiment; within those limits, size of inoculum and storage time were the least significant factors and storage environment appeared to be the most significant.

Keyphrases □ *Staphylococcus aureus* inoculation—surface of commercial tablets and capsules, effects of temperature, relative humidity, size of inoculum, and storage time on survival □ Tablet and capsule surface inoculated with *Staphylococcus aureus*—effects of temperature, relative humidity, size of inoculum, and storage time on survival □ Sterility testing, tablets and capsules inoculated with *Staphylococcus aureus*—effects of size of inoculum, storage time, and environmental conditions on survival

The necessity for sterility of parenteral products has long been recognized. NF V was the first official compendium in the United States to require sterility for such products (1). With respect to distribution and use of nonsterile pharmaceuticals, it is now recognized that

microbial contamination must be minimal; with respect to certain species, it is totally unacceptable. Kallings *et al.* (2) focused attention on microbial content of nonsterile products when they reported infections resulting from the use of contaminated medications. The Swedish National Board of Health is restricting levels of microbes that may be present in liquid and tableted preparations (3), and a policy of not allowing an excess of 100 colonies/g. for practically all medical preparations was adopted (4). Czechoslovakia adopted statutory microbiological standards for tablets (5).

Numerous studies (6–12) confirmed existence of microbial contamination in some products. Hirsch *et al.* (13) reported that 82% of 57 oral liquid products tested contained microorganisms, and other researchers (14) found more than 100 microorganisms/ml. in 40 of 489 samples of such preparations. USP XVIII now includes a statement regarding microbial contamination of nonsterile pharmaceutical products (15).

The purposes of this study were to: (a) determine the ability of solid pharmaceutical dosage forms to carry bacteria; and (b) investigate the effect of temperature, relative humidity, size of inoculum, and storage time on

Table I—Effect of Temperature, Relative Humidity, and Time of Storage on the Survival of *S. aureus* on Solid Dosage Forms[a]

| | Percentage of Cells Surviving | | | | | |
| | 3 Days | | | 7 Days | | |
Sample	Group 1[b]	Group 2	Group 3	Group 1	Group 2	Group 3
Aspirin	0[c]	0	0	0	0	0
Buffered aspirin	0	0	0	0	0	0
Sodium butabarbital	0.11	0.05	0	0.008	0.0004	0
Chewable multiple vitamins	0.12	0.08	0.04	0.01	0.001	0
Digitalis USP	5.83	1.06	0.08	2.42	0.26	0.0001
Chlorothiazide	3.51	1.26	0.25	1.75	0.45	0.10
Nasal decongestant	0	0	0	0	0	0
Meprobamate	2.12	0.53	0.003	0.93	0.073	0
Barbiturate–analgesic combination	0	0	0	0	0	0
Gelatin capsule	24.33	17.01	11.02	17.50	14.86	8.15
Magnesia hydroxide	0	0	0	0	0	0
Tolbutamide	2.25	0.73	0	1.10	0.10	0
Pentaerythritol tetranitrate	0	0	0	0	0	0
Quinidine sulfate	0	0	0	0	0	0
Magaldrate	27.67	19.12	12.16	18.33	16.23	5.07
Methocarbamol with aspirin	0	0	0	0	0	0
Antiasthmatic	0.17	0.05	0	0.01	0.003	0
Ethinamate	0.201	0.062	0	0.033	0.0001	0
Filter paper disk control	0.195	0.063	0.002	0.030	0.0045	0

[a] Each value represents the average from counts of three tablets or capsules inoculated with 3.78×10^6 bacteria. [b] Group 1, 28°, 24% relative humidity; Group 2, 33°, 10% relative humidity; and Group 3, 37°, 20% relative humidity. [c] 0 indicates no viable organisms detected.

the survival of *Staphylococcus aureus* on the surfaces of commercial products.

EXPERIMENTAL

Materials and Methods—The products (and their strength if applicable) were as follows: aspirin[1] (325 mg.), buffered aspirin[2] (325 mg.), sodium butabarbital[3] (100 mg.), chewable multiple vitamins[4], digitalis USP[5] (100 mg.), chlorothiazide[6] (500 mg.), a nasal decongestant[7], meprobamate[8] (400 mg.), a barbiturate-analgesic combination[9], a gelatin capsule[10], magnesia hydroxide[11] (325 mg.), tolbutamide[12] (500 mg.), pentaerythritol tetranitrate[13] (20 mg.), quinidine sulfate[14] (200 mg.), magaldrate[15] (400 mg.), methocarbamol with aspirin[16] (400 mg.), an antiasthmatic[17], and ethinamate[18] (500 mg.).

Survival tests were performed with *S. aureus* FDA 209[19] as the test organism, cultured in trypticase soy broth or on trypticase soy agar as appropriate. Relative humidity in the test chambers was maintained at 33, 52, or 85% (at 25°) by means of saturated solutions of magnesium chloride, magnesium nitrate, or potassium chloride, respectively (16). Potassium thiocyanate was employed as an indicator (17).

Sterilization—Products to be tested were placed in cotton-plugged erlenmeyer flasks (25 or 50 ml.) or culture tubes (25 × 130 mm.) and exposed to ethylene oxide[20] in a sterilizer[21] for 6 hr. at 55°. The efficiency of the sterilization cycle was determined by means of *Bacillus subtilis* indicator strips[22]. Sterility was rechecked after 1 week at ambient temperature by incubating control samples in 10 ml. sterile broth for 24 hr. at 37°.

Inoculation of Samples—Following the rechecking of sterility, samples were placed in humidity chambers and were inoculated after 1 week with 50 μl. of a suspension of the test organism prepared as follows:

1. Incubate broth culture 20 hr. at 37°.
2. Centrifuge.
3. Wash cells in 10 ml. sterile water.
4. Recentrifuge.
5. Resuspend in 10 ml. sterile water.

Capsules were inoculated by placing inoculum into one of the two parts, and tablets were inoculated by distributing the inoculum on the surface. All procedures were conducted in a laminar flow hood.

One, 3, and 7 days after inoculation and storage under the respective conditions, samples were placed in sufficient sterile water to make a final volume of 10 ml. They were permitted to stand for 1 hr. and then shaken. Then 50 μl. of the dispersion was streaked on agar plates; these plates were incubated at 37° for 48 hr., at which time colonies were counted.

Maintenance of sterility was verified by inoculating tablets (digitalis, chlorothiazide, and magaldrate) with sterile saline and subjecting them to the experimental conditions used in the study. To determine the basal survival rate of the microorganisms, filter paper disks[23] (12.7 mm. diameter) were inoculated because they are porous and nonbactericidal.

Contamination in Commercial Products—Commercial preparations were tested to determine the presence of microbial contaminants by transferring them aseptically from the original packages to tubes containing 10 ml. of broth. Six samples of each product were prepared. Following incubation at 37° for 24 hr., the tubes were examined for evidence of bacterial growth.

Effect of pH on Survival—To study the effect of pH on survival rates, tubes containing 10 ml. of distilled water, adjusted with acid or base to pH values of 2.5–10, were sterilized and inoculated with *S. aureus* (17.18 × 10^6 organisms). After 1 hr., 0.1-ml. samples were removed and streaked on agar plates. The plates were examined after incubation at 37° for 24 hr.

pH of Aqueous Dispersions of Tablets—Triplicate samples of products were placed in sufficient water to make 30 ml. After the tablets disintegrated, the pH of the resulting dispersions was measured.

Statistical Analysis—The data were analyzed using a two-way factorial analysis of variance. The variables involved were time

[1] Lot No. 216, Bayer Co.
[2] Bufferin.
[3] Lot No. ML 7667, Butisol.
[4] Lot No. 7629 ML, Chocks.
[5] Lot No. 293-209, Lederle.
[6] Lot No. 693, Diuril.
[7] Lot No. 1366-26, Dristan.
[8] Lot No. 1710839, Equanil.
[9] Lot No. 116 R 1359, Fiorinal.
[10] Lot No. LC 6011 C, Eli Lilly & Co.
[11] Lot No. 8M575, Squibb.
[12] Lot No. 1H P35 IO, Orinase.
[13] Lot No. 0277 PO 29 B, Peritrate.
[14] Lot No. 5DD53A, Eli Lilly & Co.
[15] Lot No. A3503LL, Riopan chewable.
[16] Lot No. 71 1723 4, Robaxisal.
[17] Lot No. 0174P1049A, Tedral.
[18] Lot No. 50D72A, Valmid.
[19] Supplied by the Department of Microbiology, University of Georgia.
[20] PennGas, Pennsylvania Engineering Co., Philadelphia, Pa.
[21] Cryotherm Gas Sterilizer, American Sterilizer Co., Erie, Pa.
[22] Spordex, American Sterilizer Co., Erie, Pa.

[23] No. 740-E, Carl Schleicher and Schuell Co., Keene, N. H.

Table II—Effect of Size of Inoculum, Relative Humidity, and Time of Storage on the Survival[a] of *S. aureus* on Solid Dosage Forms Stored at 25°

Sample		Percentage of Cells Surviving								
		1 Day			3 Days			7 Days		
		Group 4[b]	Group 5	Group 6	Group 4	Group 5	Group 6	Group 4	Group 5	Group 6
Sodium butabarbital	(L)	2.11	0.96	0.415	0.18	0.087	0.022	0.012	0.003	0.0004
	(H)	1.30	0.61	0.29	0.105	0.048	0.007	0.008	0.0007	0.0001
Chewable multiple vitamins	(L)	1.11	0.522	0.22	0.175	0.081	0.017	0.015	0.003	0
	(H)	0.77	0.371	0.160	0.115	0.051	0.009	0.009	0.0006	0.0001
Digitalis USP	(L)	12.67	7.025	3.221	5.73	3.41	0.842	2.28	0.717	0.093
	(H)	6.31	4.09	1.93	3.57	1.83	0.47	1.23	0.256	0.010
Chlorothiazide	(L)	6.51	3.51	1.17	5.27	1.33	0.42	1.93	0.09	0.007
	(H)	3.22	1.84	0.81	2.70	0.617	0.15	0.998	0.025	0.001
Meprobamate	(L)	10.36	6.12	2.317	2.67	1.71	0.622	1.052	0.123	0.047
	(H)	4.171	3.09	1.23	1.53	0.81	0.42	0.875	0.044	0.008
Gelatin capsule	(L)	30.26	19.11	6.05	25.81	8.93	2.12	16.33	2.49	0.295
	(H)	14.39	8.12	3.11	12.57	4.735	0.875	9.425	0.745	0.091
Tolbutamide	(L)	6.83	3.94	1.27	2.41	1.11	0.37	1.26	0.07	0.004
	(H)	8.54	2.11	0.92	1.33	0.42	0.124	0.834	0.013	0.0009
Magaldrate	(L)	34.57	20.33	7.35	32.18	9.42	2.45	17.45	1.625	0.32
	(H)	18.71	9.94	4.285	15.45	3.77	0.937	11.375	0.641	0.105
Antiasthmatic	(L)	0.83	0.49	0.193	0.215	0.093	0.024	0.023	0.005	0.0004
	(H)	0.65	0.30	0.117	0.119	0.06	0.009	0.011	0.0009	0.0001
Ethinamate	(L)	0.717	0.422	0.15	0.238	0.125	0.028	0.035	0.009	0.0007
	(H)	0.59	0.26	0.09	0.145	0.074	0.02	0.015	0.002	0.0002
Filter paper disk control	(L)	1.13	0.64	0.33	0.235	0.105	0.025	0.035	0.007	0.0005
	(H)	1.025	0.572	0.191	0.133	0.06	0.01	0.015	0.001	0.0001

[a] Each value reported represents the average from counts of two tablets or capsules. (L) = 1.47×10^6 bacteria/sample. (H) = 13.57×10^6 bacteria/sample. [b] Group 4, 33% relative humdity; Group 5, 52% relative humidity; and Group 6, 85% relative humidity.

(three categories), level of inoculum concentration (two categories), and environmental storage conditions (three categories). The *F*-distribution was used as the test of significance in the analysis of variance.

RESULTS

The survival of *S. aureus* on solid dosage forms under conditions where storage time, temperature, and relative humidity were varied is reported in Table I. The data reveal decreased survival with increased temperature and storage time. *S. aureus* did not survive on eight samples (aspirin, the nasal decongestant, the barbiturate-analgesic combination, methocarbamol with aspirin, magnesia hydroxide, buffered aspirin, quinidine, and pentaerythritol tetranitrate tablets). There was a high frequency of survival on the gelatin capsules (8%) and magaldrate (5%) after 7 days under conditions that resulted in no survivors on 15 of the products.

Table II shows the percent survival of *S. aureus* after storage at a constant temperature of 25° when relative humidity and size of inoculum varied. Decreased survival rates were noted when relative humidity, storage time, and inoculum concentration were increased.

Tests on unsterilized pharmaceuticals revealed contamination of 12. Contaminants were not identified. Bacterial growth was not observed in the tubes containing aspirin, buffered aspirin, sodium butabarbital, the nasal decongestant, magnesia hydroxide, and methocarbamol with aspirin.

The pH of aqueous dispersions of the tablets ranged from 2.7 to 9.8. *S. aureus* did not survive in water outside the pH range of 4.0–8.6. Maximum survival was observed in the pH range of 7.0–8.6.

DISCUSSION

Sterility is not a requirement in official compendia for oral pharmaceutical dosage forms. However, contamination may occur during manufacturing, packaging, and handling by the consumer. This causes concern, since some dosage forms, if stored in favorable environments, can serve as substrates for microorganisms. Not all of the dosage forms studied supported bacterial (staphylococcal) contamination under the experimental conditions, suggesting that some pharmaceuticals are less likely than others to support bacterial survival (or at least survival of staphylococci).

Many factors determine whether a microorganism will survive on a solid dosage form. Active ingredient and formulation additives may have a profound effect on bacteria; in certain formulations, they may act as bactericidal agents. Environmental conditions such as humidity, temperature, and absence or presence of oxygen may give rise to unfavorable conditions for survival of bacteria. Other factors involved may be size of inoculum and length of storage (18).

Using aqueous solutions adjusted to varying pH's with acid or base, it was found that *S. aureus* did not survive except in the pH range of 4.0–8.6. Because the pH of aqueous dispersions of aspirin, the nasal decongestant, the barbiturate–analgesic combination, methocarbamol with aspirin, and magnesia hydroxide was outside the range of 4.0–8.6, pH was considered a major factor for *S. aureus* not surviving. However, lack of survival cannot be explained solely on the basis of pH since the other three tablets (buffered aspirin, quinidine, and pentaerythritol tetranitrate) did not permit survival even though aqueous dispersions were in the favorable pH range of 4.0–8.6. The formulation ingredients may be the reason for zero survival of *S. aureus* on these tablets, since bacteria survived on the filter paper disk control under the same experimental conditions.

At high relative humidities, the survival rate was reduced (Table II). A thin aqueous film may form on the surface of the solid dosage forms and, as the humidity increases, this aqueous film formation should increase. Not only would more water be available for the organism, but additional quantities of the soluble ingredients would be dissolved in the film and a greater bactericidal action would be exerted. The existence of a critical moisture content at which a bacterial cell becomes more susceptible to toxic agents has been postulated (19).

Table I shows that there were fewer survivors at 37° than at 28° where the relative humidities were similar. This may be due in part to increased solubility of tablet ingredients in the surface film at the higher temperature and the fact that the bactericidal action of chemical agents is greater at higher temperatures (18). Consequently, there would be a greater bactericidal effect on *S. aureus*.

The frequency of survivors on nine samples after storage for 7 days at 25° and 33% relative humidity was 100–1000-fold higher than on the control filter paper disks, indicating that ingredients in the samples protected the *S. aureus*.

High and low concentrations of inoculum were used to determine the effect on survival rates, because some medications may be more contaminated than others. Differences between percent survivals were noted; in general, the highest survival rate was observed with the lower inoculum concentrations. The differences were not statis-

tically significant, and no explanation can be offered for these results.

The size of inoculum and storage time (within the limitations of the experiment) were less significant factors. Storage conditions appeared to be the most significant factor.

Numerous variables were inherent in the study, and the data are inconclusive. Future studies may provide data to explain the differences in survival rates of *S. aureus* on solid dosage forms.

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Physical–Chemical Properties of Substituted Amides in Aqueous Solution and Evaluation of Their Potential Use as Solubilizing Agents

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Abstract ☐ The tetramethyl-substituted amides of pimelamide, suberamide, azelamide, and sebacamide markedly enhance the solubility of glutethimide in aqueous solution. Partition studies, surface tension measurements, and light-scattering measurements strongly suggest that the amides are associating at infinitely dilute concentrations and further aggregation of these associated molecules occurs with the possible formation of micelles at concentrations slightly higher than that observed for surfactants. The CMC's were identified at 0.41, 0.20, 0.031, and 0.11 *M* for pimelamide, suberamide, azelamide, and sebacamide, respectively. The solubility of glutethimide was increased significantly above the critical concentrations and, from the nature of the solubility curves, a micellar type of solubilization appears to be dominant.

Keyphrases ☐ Amides, tetramethyl substituted—partitioning, surface tension, and light-scattering measurements in aqueous solution, potential solubilizing agents (solubilization of glutethimide) ☐ Pimelamide, tetramethyl, as potential solubilizing agent—partitioning, surface tension, and light-scattering measurements in aqueous solution, solubilization of glutethimide ☐ Suberamide, tetramethyl, as potential solubilizing agent—partitioning, surface tension, and light-scattering measurements in aqueous solution, solubilization of glutethimide ☐ Azelamide, tetramethyl, as potential solubilizing agent—partitioning, surface tension, and light-scattering measurements in aqueous solution, solubilization of glutethimide ☐ Sebacamide, tetramethyl, as potential solubilizing agent—partitioning, surface tension, and light-scattering measurements in aqueous solution, solubilization of glutethimide ☐ Solubilizing agents, potential—tetramethyl-substituted amides ☐ Glutethimide solubility—effect of tetramethyl-substituted amides

The formulation of a suitable dosage form for administration *via* the oral, parenteral, and topical routes is often a problem with drugs that have only limited water solubility. For oral administration, a solution would preclude the requirement for dissolution as in the case of suspensions or solid dosage forms and facilitate passage of the drug across the lipid membrane. For topical preparations, a homogeneous system would facilitate percutaneous absorption; for parenteral administration, a solution is generally desirable, especially *via* the intravenous route.

Nonaqueous solvents are often combined with water to dissolve a sparingly soluble drug even for injectable preparations (1), and the solvent selected must be free of toxic and irritating effects and not give rise to pharmacological responses. Two-component solvent systems