TABLE I.
Toxicity of Solutions of Cocaine Hydrochloride.*

Age of solution	Rabbits	Deaths	Mortality rate %
Fresh	76	15	19.7
1 wk	87	25	28.7
1 mo	36	9	25.0
3½ ''	10	1	10.0

* The drug was injected intravenously in 10 seconds in the dosage of 10 mg per kilo of body weight, a 1% solution being used.

grading of convulsions seemed impossible (Table I). It will be seen that there was not a demonstrable difference in the toxicity of the solutions of various ages. There was no evidence that the sex or the weight of the rabbits had any influence on the mortality rate. Neither was there any evidence that the fresh solution of cocaine hydrochloride became more toxic during the hour-long period that it was being used.

These results do not support the clinical impression that fresh solutions of cocaine hydrochloride are less toxic than older ones. However, it has been shown that sensitivity to cocaine hydrochloride varies among members of the animal kingdom, the monkey being much more sensitive than the rabbit.[8] It seems probable that the human being is even more sensitive. If this is true, then it seems possible that small differences in toxicity which could not be detected in the rabbit might be of real significance in human beings. Therefore, even though the older solutions of cocaine hydrochloride did not cause a higher death rate in rabbits, it should not be concluded that old solutions of cocaine are entirely safe for human beings. In view of the clinical reports cited in the earlier part of this paper, it would seem wise to use fresh solutions of cocaine exclusively in clinical work.

Summary. The toxicity of solutions of cocaine hydrochloride of various ages (less than one hour, one week, one month and 3½ months) was studied in rabbits, the drug being given intravenously in the dosage of 10 mg per kilo of body weight. The mortality rates following injections of solutions of cocaine of various ages showed no significant differences.

[8] Tatum, A. L., and Collins, A. H., *Arch. Int. Med.*, 1926, **38**, 405.

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Protection of Dry Bacteria by Fat Against Cationic Detergents.

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Klarmann and Wright[1] have shown that quaternary ammonium compounds cause an agglutination of bacteria and precipitate them against the walls of the test tube. This causes a very coarse and irregular distribution and explains the irregular results obtained by every investigator trying to evaluate the germicidal efficiency of these compounds.

It is not clear, however, why the bacteria in these clumps die more slowly than those which remain singly in suspension. In order to prove whether these detergents are strongly adsorbed on the surfaces of bacteria, 1 g of baker's yeast was suspended in 100 ml of a 100 ppm solution of Zephiran (dimethylbenzyl alkyl ammonium chloride, also known as Roccal), the yeast removed by centrifugation, and the surface tension of the clear solution measured by the drop number method (Table I.) Nearly 20 ppm of the Zephiran had been adsorbed on the cells. This amount indicates a multimolecular adsorption layer. Bentonite particles in 1000 ppm Zephiran

[1] Klarmann, E. G., and Wright, E. S., *Soap and San. Chem.*, 1946, **22**, 125.

TABLE I.
Adsorption of Zephiran on Various Surfaces.

Solution	Surface tension
Water	72.8 dynes
50 ppm Zephiran	66.5
80 " "	62.5
100 " "	59.2
Same + 1.3% filter paper	62.2
Same + 1% bakers yeast	63.4
1000 ppm Zephiran	37
Same + 5% bentonite	73

solution increased noticeably in volume, suggesting thick adsorption layers. Such layers may retard the diffusion of dissolved molecules to the center of a clump of bacteria.

However, another fact must also be included into any explanation, namely the observation by Miller, Abrams, Huber, and Klein[2] that hands dipped in 1% Zephiran solution remain apparently sterile for about 2 hours through an imperceptible film, while the bacteria of the skin, under this film, are still alive when the film is disrupted several hours later.

This observation can be explained by assuming that the adsorbed Zephiran molecules or ions are oriented, the inactive, harmless organic end directed towards the skin and the germicidal NOH group towards the outside. The organophilic skin attracts the long organic chains, and thus, the adsorption film has one harmless side and one toxic side.

To test this explanation, experiments were made with 2 different surfaces, namely fat which is organophilic, and 25% gelatin which is hydrophilic. About 1 to 2 g butterfat of high melting point were pipetted into test-tubes, and after solidification, 0.1 ml of a Staphylococcus culture was put upon the fat surface. When the culture was perfectly dry (in a vacuum), 2 to 5 ml of a 1% Zephiran solution were poured on the dry bacteria. After various exposure times, the disinfectant was poured off, the entire tube was washed once with 50 ppm Duponol (a good antidote to Zephiran), then twice with 10 ppm Duponol, and finally, broth containing 5 ppm Duponol was poured into the tube. The fat surface was then scraped with a bent platinum wire to remove as many bacteria as possible, the tube was shaken violently, and 1 ml and 0.01 ml of the broth was plated. The counts thus obtained are extremely inaccurate because bacteria may not all be removed, or may become imbedded in the fat. The chance of contamination by the repeated washing is increased. The recovery of bacteria dried on fat and treated with water gave between 200 and 20,000 colonies. If only very few colonies are found, and not consistently, it suggests a chance contamination while larger numbers are good proof of survival.

[2] Miller, B. F., Abrams, B., Huber, D. A., and Klein, M., PROC. SOC. EXP. BIOL. AND MED., 1943, **54**, 174.

TABLE II.
Survivors of *Staphylococcus aureus* Exposed to Zephiran Solution in Presence of Various Surfaces.

Surface	Butterfat		
Disinfectant	1% Zephiran		
Bacteria	Dried I	Dried II	Moist II
0.5 hr	12,100	—	—
1	4,000	170	—
1.5	50,000	—	—
4	—	190	0
20	—	30	0

Surface	25% gelatin				
Disinfectant	1% Zephiran	0.1% Zephiran		0.01% Zephiran	
Bacteria	dry	dry	moist	dry	moist
0.5 hr	0	0	0	1:590	9,700
1	0	0	—	0	—
1.5	0	0	0	900	0
4	0	0	—	0	—

TABLE III.
Survivors of *Staphylococcus aureus* Exposed to Disinfectants in Presence of Various Surfaces.

Surface	Vaspar		Fat		Glass	
Bacteria	dried	moist	dried	moist	dried	moist
	Disinfectant: 8% formaldehyde					
0.5 hr	0	0	261	—	0	4
1	0	0	14	0	0	1
2	900	1	0	—	—	0
	Disinfectant: 1% ceepryn					
0.5	4,400	4	—	0	0	1
1	3,500	0	24,700	42	0	0
2	—	23	—	—	—	—
3.5	24	—	0	—	0	0
7	7	—	22	—	0	—
22	0	—	0	—	—	—

The procedure with gelatin was similar, except that finally, the gelatin was melted in the broth at 40°, and plated directly. The result is shown in Table II. Bacteria dried on fat survived the powerful disinfectant for 20 hours, while they died within half an hour when not dried. By the standard technic, 50 ppm kill *Staphylococcus aureus* in 10 minutes. Bacteria dried on gelatin did not survive half an hour. Evidently the 2 surfaces affected the bacteria differently.

Table III gives the results of a parallel experiment with Ceepryn (cetyl pyridinium chloride), and with formaldehyde. Fat exerted a definite protection against formaldehyde, but not as pronounced as against Ceepryn. Vaspar (vaseline-paraffine mixture) as a pure hydrocarbon is even more organophilic than fat. The glass surface offered no protection. Bacteria dried on sand were also killed as easily as without sand.

Mudd and Mudd[3] have shown that bacteria are drawn to the interface between oil and water and usually remain there. Only the acid-fast bacteria are drawn into the oil phase. However, Jensen[4] states: "If sodium oleate in 0.05% solution is added to the water, every species and kind of microbes pass into the oil." A Zephiran solution will probably exert an influence similar to that of sodium oleate. Although the fat was solid, it is possible that a very thin film of fat covered some of the dry bacteria. Or perhaps, the surface of bacteria is so changed by drying that it becomes organophilic and becomes saturated with fat if that is present. Such cells would be protected against quaternary ammonium compounds because they would adsorb the harmless end of the molecule.

Summary. The adsorption of quaternary ammonium compounds on cell surfaces has been proved experimentally.

The formation, on hands, of a film of cationic detergent of which only the outside is germicidal, has been explained as due to an oriented adsorption of the detergents on an organophilic surface, such as fat or paraffin.

[3] Mudd, S., and Mudd, E. B. H., *J. Exp. Med.*, 1924, **40**, 659.

[4] Jensen, L. B., *Microbiology of Meats*, Garrard Press, 1945, 286.