made to determine the relation, if any, of "mad itch" virus, to a rarely occurring but fatal bovine pruritic syndrome in Illinois. Pursuant to this inquiry, tissues from suspected natural cases have been examined for the presence of a filtrable virus. Notwithstanding the clinical resemblance of the disease reported by Illinois veterinarians to the "mad itch" syndrome in cattle, the virus of the disease was only recently identified in Illinois.

In February, 1940, a well-nourished Hereford steer (24333) weighing approximately 1,000 pounds, displaying a syndrome resembling that of pseudorabies, was brought to the university laboratory for examination. Twenty-four hours later, the animal, in a moribund condition, was sacrificed for autopsy. No gross pathologic lesions were noted other than self-inflicted lacerations of the skin and subcutaneous tissue in the left dorso-lumbar region.

Anaerobic and aerobic cultures of the heart blood, brain and dorsal spinal cord proved negative. Physiological sodium chloride suspensions of the brain and spinal cord as well as urine collected aseptically from the bladder were injected subcutaneously into a series of 12 rabbits and 12 guinea pigs. All inoculated rabbits and guinea pigs, with the exception of those receiving the dorsal spinal cord tissue, remained healthy. Both rabbits and one of the two guinea pigs receiving subcutaneous injections of the spinal cord tissue succumbed in 80 to 108 hours. Preceding death, the inoculated rabbits and guinea pig showed markedly increased respirations and evidence of intense pruritus near the point of inoculation. A filtrable agent (Berkefeld N) which reproduced the disease in rabbits and a calf, was demonstrated in the combined spinal cord tissue of the steer and the spinal cord tissue of rabbits that succumbed following inoculation. In further confirmation of the nature of the filtrable agent the chorioallantoic membrane of twelve-day chick embryos inoculated (Goodpasture method[4]) with the bacteriologically sterile spinal cord tissue suspension resulted in abundant gross lesions similar to those reported by Glover.[5] Serum neutralization tests of the steer spinal cord virus (24333) were conducted by Shope.[6] The results of his immunological tests support the preliminary deduction that the virus is that of pseudorabies or "mad itch."

ROBERT GRAHAM
C. C. MORRILL
L. E. BOLEY

UNIVERSITY OF ILLINOIS

[3] A. Aujeszky, *Centr. Bakt. I. Abt.*, 32: 353, Orig., 1902.

[4] E. W. Goodpasture, A. M. Woodruff and J. G. Buddingh, SCIENCE, 74: 371, 1931; *Amer. Jour. Path.*, 8: 271, 1932.

[5] R. E. Glover, *British Jour. of Exp. Path.*, 20: 150, 1939.

[6] R. E. Shope, personal communication.

INHIBITION OF BACTERIAL METABOLISM BY SYNTHETIC DETERGENTS[1]

THE bactericidal and lytic action of soaps and certain naturally-occurring detergents such as bile salts has been recognized for some years. Recently, the demands of industry for wetting agents and detergents to meet a variety of special purposes have led to a very rapid commercial development of these compounds. More than a thousand wetting agents and detergents have been patented in the past decade. In 1935 Domagh[2] reported that the quaternary ammonium detergent, Zephiran [alkyl (C_8 to C_{18}) dimethyl benzyl ammonium chloride], possesses germicidal properties, and he recommended its use for disinfection of skin surfaces. Katz and Lipsitz[3] found that one cationic and three anionic synthetic wetting agents inhibited the growth of Mycobacterium smegmatis. Cowles[4] and Birkeland and Steinhaus[5] made the interesting observation that alkyl sulfates selectively inhibit the growth of gram-positive organisms.

A very marked inhibitory action of Zephiran on the respiration and glycolysis of pure cultures of microorganisms associated with dental caries has been demonstrated by Miller, Baker and Harrison.[6] Also, as shown by Miller, Muntz and Bradel,[7] this compound penetrates the dense matrix of human dental plaque material both *in vitro* and *in vivo*, and inhibits the metabolism of the mixed flora.

In the present communication we report some results obtained in a study of the effects of a variety of synthetic wetting agents and detergents on bacterial metabolism. The metabolism of freshly prepared suspensions of microorganisms was measured in the usual manner in the Warburg manometric apparatus in the presence of either phosphate or bicarbonate buffer at pH 7.3. The vessels contained 15 to 25 billion cells in a volume of 3 cc. Six gram-positive and six gram-negative organisms were studied. The detergents and wetting agents have been classified as *cationic* or *anionic*. Thus Zephiran, typical of the cationic compounds, ionizes with the long-chain hydrophobic group in the cation:

$$[(R_1R_2R_3R_4)N]^+ + (Cl^-)$$

Sodium cetyl sulfate, typically anionic, ionizes with the hydrophobic group in the anion as follows: $(C_{16}H_{33}\text{–}O\text{–}SO_3)^- + (Na)^+$.

Several types of *cationic* detergents have been investigated. These may be grouped as follows: (a) *quater-*

[1] From the Walter G. Zoller Memorial Dental Clinic and the Department of Medicine, University of Chicago.

[2] G. Domagh, *Deutsche Med. Wochenschr.*, 61: 829, 1935.

[3] J. Katz and A. Lipsitz, *Jour. Bact.*, 30: 419, 1935; 33: 479, 1937.

[4] P. B. Cowles, *Yale Jour. Biol. Med.*, 11: 33, 1938.

[5] J. M. Birkeland and E. A. Steinhaus, *Proc. Soc. Exper. Biol. and Med.*, 40: 86, 1939.

[6] B. F. Miller, Z. Baker and R. W. Harrison, *Proc. Soc. Exper. Biol. and Med.*, 42: 705, 1939.

[7] B. F. Miller, J. Muntz and S. Bradel, in press.

nary ammonium compounds containing aromatic or alkyl-phenyl radicals: Zephiran [alkyl (C_8 to C_{18}) dimethyl benzyl ammonium chloride]; Triton K–12 (chiefly lauryl dimethyl benzyl ammonium chloride); Triton K–60 (chiefly cetyl dimethyl benzyl ammonium chloride); Hydrocide (alkyl hydroxy benzyl dimethyl ammonium phosphate); (b) *quaternary ammonium compounds containing only aliphatic groups:* Dupont Retarder LA (stearyl trimethyl ammonium bromide); Damol [$(CH_3)_2(C_{12}H_{25})$ N (Br)–CH_2–CHOH–CH_2–(Br) N ($C_{12}H_{25}$) (CH_3)$_2$]; Emulsol-605 [$C_{11}H_{23}$–COO–C_2H_4–NH–CO–CH_2–N$(CH_3)_3$CI];[8] (c) *quaternary ammonium salts containing heterocyclic nitrogen:* CēPryn chloride[9] (cetyl pyridinium chloride); Emulsol-660 B (lauryl pyridinium iodide); (d) *non-quaternary compounds:* only one such detergent was available for our studies, Emulsol-606, which is the lauryl ester of glycine hydrochloride.[8] It was found that all of these compounds inhibited the metabolism of the organisms almost completely at a concentration of 1:3000. Most of the compounds were equally effective at 1:30,000. In a few cases a marked effect on bacterial metabolism was noted at concentrations as low as 1:60,000. *These cationic detergents inhibited the metabolism of both gram-positive and gram-negative microorganisms to the same degree.*

The effects of the following *anionic* detergents were studied: sodium cetyl sulfate; Duponol LS (sodium oleyl sulfate); Igepon A (R–COO–CH_2–CH_2SO_3Na) and Igepon T [R–CO–N(CH_3)–$(CH_2)_2SO_3Na$]; Tergitol 7 (sodium alkyl sulfate, alkyl = 3,9 diethyltridecanol-6); Drene (triethanolamine lauryl sulfate); Triton W-30 (sodium salt of alkyl phenoxy ethyl sulfonate); Triton 720 (sodium salt of alkyl phenoxy dialkoxy sulfate); Nopcocastor V (sulfonated castor oil); sodium taurocholate. In contrast to the results with the cationic compounds, it was found that few of the anionic wetting agents inhibited the metabolism of either gram-positive or negative organisms appreciably at a concentration of 1:30,000. Only one, Tergitol 7, was able to inhibit completely the six gram-positive organisms at a dilution of 1:3000. At this concentration, the other anionic compounds inhibited some, but not all of the *gram-positive* bacteria. There was seldom any significant effect by these compounds on the metabolism of *gram-negative* organisms at the 1:3000 dilution.

It may be concluded that the *cationic* type of detergent is a more general inhibitor of bacterial metabolism than the *anionic.*

Variations in the pH of the buffer medium caused striking differences in inhibitory action. It was found that the effect of the cationic compounds increased progressively as the pH was shifted toward the alkaline side, whereas inhibition by the anionic types increased with a shift toward the acid side.

A series of pure alkyl sulfates ranging from C_8 to C_{18} was studied on some gram-positive organisms. It was found that the C_{12} and C_{14} (lauryl and myristyl) compounds gave the maximum inhibitory effects.

We have found that Damol, Emulsol-605 and Emulsol-606 possess a reasonably low toxicity for mice by intraperitoneal injection, and that they produce little or no irritation in the rabbit eye at concentrations of 1:500 to 1:1000. Of these, the lauryl ester of glycine hydrochloride (Emulsol-606) is the least toxic. The protective action of these compounds towards experimentally-induced infections is being studied.

The experiments described here will be published in full elsewhere.

BENJAMIN F. MILLER
ZELMA BAKER
THE UNIVERSITY OF CHICAGO

SCIENTIFIC APPARATUS AND LABORATORY METHODS

ETHYL METHACRYLATE AS A MOUNTING MEDIUM FOR EMBRYOLOGICAL SPECIMENS

A SUITABLE method of mounting various embryological specimens such as small mammalian embryos, older chick embryos and amphibian eggs and embryos has always been a problem for the teacher of embryology. These objects were too large for balsam mounts; if they could be mounted in balsam, they dried very slowly and were very easily broken. The only satisfactory method has been to study these objects in dishes of a preservative such as alcohol. Experiments carried out recently in this laboratory have shown that permanent preparations of splendid optical qualities can be made by embedding these objects in one of the clear plastics, ethyl methacrylate. The manner in which this is accomplished is described below.

The unpolymerized ethyl methacrylate monomer can be obtained from the Rohm and Haas Chemical Co., Philadelphia, Pa., at a cost of about $6.00 per gallon. This material is shipped with an inhibitor, hydroquinone, which must be removed before polymerization. This inhibitor is removed by washing a sample of the monomer (300 cc samples are used in this laboratory) four or five times with a 5 per cent. KOH solution.

[8] We are indebted to Messrs. A. K. Epstein and B. R. Harris, chemists of the Emulsol Corporation, for suggesting that bactericidal compounds of the type 605 and 606 would possess low local and systemic toxicity.

[9] The toxicity and germicidal action of cetyl pyridinium chloride have been investigated by R. S. Shelton *et al.* (Abstracts 99th Meeting of the American Chemical Society, April, 1940).