ous dose revealed that to achieve an effect with tubocurarine electrophoresis only a maximum of 0.011 mg tubocurarine need reach the muscle.

Thus, further evidence is adduced that penetration of substances through the skin by electrophoresis is limited.

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## *In vitro* Effect of Certain Antibacterial Agents on Organisms Encountered in Bovine Mastitis.*

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In screening materials to learn which gave promise in the treatment of bovine mastitis, the *in vitro* effect of a number of antibiotic substances and sulfonamides was determined on some of the bacteria frequently associated with cases of bovine mastitis.

Some information is already available in the literature relative to the materials employed. Salle and Jann[1] reported that subtilin was bacteriostatic in high dilutions and bactericidal in lower dilutions for gram positive organisms, including *Mycobacterium tuberculosis*. Buggs and co-workers,[2] working with streptomycin *in vitro* found a great range in susceptibility of different strains of the same organism.

Goetchius and Lawrence[3] found that 3'-5' dibromo sulfanilanilide *in vitro* was effective in high dilution against *Streptococcus agalactiae, Streptococcus viridans,* and *Brucella abortus*. Schweinberg and Yetwin[4] stated that sulfamethazine *in vitro* was more bactericidal and bacteriostatic than sulfadiazine or sulfamerazine against *Eberthella typhosa, Escherichia coli,* and certain Salmonella. Francis *et al.*[5] reported that 4, 4' diaminodiphenyl sulfone (hereafter designated sulfone) showed promise against *Str. agalactiae* infections in the chick embryo and mouse. Burbaum and others[6] found that sulfadiazine was slightly inhibitory against *Corynebacterium diphtheriae.*

*Methods.* The organisms used in these experiments were *Str. agalactiae, Br. abortus, Pseudomonas aeruginosa, E. coli,* a coagulase-positive *Staphylococcus aureus,* and *Corynebacterium pyogenes. Salmonella typhimurium* was employed as a representative of the Salmonella.

A culture of each of the above organisms was first grown in tryptose broth for 24 hours at 37°C. Inocula for the actual tests were prepared from such broth cultures. The *C. pyogenes* broth culture was used undiluted while the others were diluted 1 to 100 in broth. Various concentrations of each of the antibacterial agents were prepared in 10 ml of sterile skimmed milk to which brom cresol purple was added as an indicator, and these tubes were inoculated with 0.2 ml of the suspensions of the organisms. Those cultures producing no visible change in milk were streaked on blood agar or nutrient agar slants to determine the extent to which the culture had grown in the presence of the agent. Thus the tests were carried out in skimmed milk

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1 Salle, A. J., and Jann, G. J., *J. Bact.*, 1946, **51**, 592.

2 Buggs, C. W., Bronstein, B., Hirshfeld, J. W., and Pilling, M. A., *J.A.M.A.*, 1946, **130**, 64.

3 Goetchius, G. R., and Lawrence, C. A., *J. Lab. and Clin. Med.*, 1946, **31**, 336.

4 Schweinberg, F. B., and Yetwin, I. L., *J. Bact.*, 1946, **49**, 193.

5 Francis, J., Peters, J. M., and Davies, O. L., *J. Comp. Path.*, 1947, **57**, 162.

6 Burbaum, L., Nenner, N., and Dolgopol, V. B., *J. Bact.*, 1947, **53**, 507.

TABLE I.
Dilutions of Subtilin Necessary for Bactericidal and Bacteriostatic Action After 72 Hours Incubation.

| Effect | *S. aureus* | *Str. agalactiae* | *C. pyogenes* |
|---|---|---|---|
| Bactericidal | 1:80,000 | 1:80,000 | 1:100,000 |
| Bacteriostatic | 1:160,000 | 1:160,000 | 1:200,000 |

Subtilin lot 205-0, potency equivalent to 200, was obtained through the kindness of Dr. Hans Lineweaver, Western Regional Laboratory, Albany, Calif.

TABLE II.
Effect of Streptomycin on the Various Organisms After 72 hours Incubation, Reported as Units per ml.

| Effect | *S. aureus* | *Str. agalactiae* | *C. pyogenes* | *S. typhimurium* | *E. coli* | *Br. abortus* | *Ps. aeruginosa* |
|---|---|---|---|---|---|---|---|
| Bactericidal | 50 | 50 | 25 | 500 | 62.5 | 25 | 1000 |
| Bacteriostatic | 25 | 25 | 12.5 | 250 | — | 12.5 | — |

Streptomycin, lot No. 544, containing 573 units/mg, was obtained through the courtesy of Merck & Co., Rahway, N.J.

TABLE III.
Effect of Sulfonamides on the Organisms After 24 Hours Incubation.

| Sulfonamide | Mg % conc. | *S. aureus* | *Str. agalactiae* | *C. pyogenes* | *S. typhimurium* | *E. coli* | *Br. abortus* | *Ps. aeruginosa* |
|---|---|---|---|---|---|---|---|---|
| Control | | 3+ | 3+ | 3+ | 3+ | 3+ | 3+ | 3+ |
| 3'-5'-dibromo-sulfanilanilide | 8 | 2+ | 3+ | 3+ | 3+ | 3+ | 3+ | 3+ |
| Sulfamethazine | 8 | 3+ | + | + | 3+ | — | — | 3+ |
| | 1.6 | 3+ | 2+ | + | 3+ | + | 2+ | 3+ |
| Sulfapyridine | 8 | 2+ | — | — | 2+ | ± | + | ± |
| | 1.6 | 3+ | + | 3+ | 3+ | + | + | 2+ |
| Sulfone | 8 | 2+ | ± | — | 3+ | 2+ | + | 3+ |
| | 1.6 | 3+ | + | 3+ | 3+ | 3+ | 2+ | 3+ |
| Sulfadiazine | 8 | 2+ | ± | — | 2+ | ± | — | — |
| | 1.6 | 3+ | + | 3+ | 3+ | ± | + | — |
| Succinyl sulfanilamide | 8 | 3+ | 3+ | 3+ | 3+ | 3+ | 3+ | 3+ |
| Sulfamerazine | 8 | 2+ | — | — | 3+ | — | — | ± |
| | 1.6 | 3+ | ± | — | 3+ | ± | + | 2+ |

— Complete inhibition of growth in milk, organisms not killed.
± Slight growth of organisms in milk.
+, 3+ Relative amounts of growth.

to provide somewhat the same medium in which the organisms are found growing in the bovine udder. The organisms were diluted to the approximate number likely to be found in mastitis.

Likewise, the various antibacterial agents were diluted to the approximate level which could be attained for a period of 10 to 12 hours after intramammary infusion in cows. The agents tested were subtilin, streptomycin, 3'-5' dibromo sulfanilanilide, sulfamethazine, sulfapyridine, sulfone, sulfadiazine, succinyl sulfanilamide, sulfamerazine, disodium sulfone, and sodium sulfamethazine. The lowest concentration which prevented visible growth was termed the bacteriostatic concentration: that which killed all organisms, the bactericidal concentration.

*Results.* In Table I the results of studies on subtilin are compiled. *S. aureus* and *Str. agalactiae* were slightly more resistant than *C. pyogenes*. Table II shows the bacteriostatic and bactericidal concentration of streptomycin for the seven organisms. *Ps. aeruginosa* and *S. typhimurium* were much more resistant than the other organisms. In Table III the effects of various sulfonamides on the different organisms are shown. The 3'-5' dibromo sulfanilanilide and succinyl sulfanilamide were not inhibitory in the amounts used; their bacteriostatic concentration is more than 8 mg %. Sulfamerazine and sulfadiazine

TABLE IV.
Effect of Disodium Sulfone on Various Organisms After 72 Hours Incubation.

| Mg % conc. of sulfone | *S. aureus* | *Str. agalactiae* | *C. pyogenes* | *S. typhimurium* | *E. coli* | *Br. abortus* | *Ps. aeruginosa* |
|---|---|---|---|---|---|---|---|
| 2270 | — | — | — | ± | ± | — | ± |
| 1135 | 0 | 0 | 0 | 0 | 0 | 0 | 3+ |
| 227 | ± | ± | ± | 2+ | ± | + | 0 |
| 114 | 2+ | 0 | + | 3+ | + | 2+ | 0 |
| 23 | 0 | ± | 0 | 0 | 0 | 0 | 0 |
| 11 | 0 | + | 0 | 0 | 0 | 0 | 0 |
| Control | 3+ | 3+ | 3+ | 3+ | 3+ | 3+ | 3+ |

0—No determination run.
— No growth, culture killed.
± Slight growth with no visible change in milk.
+, 3+ Relative amount of growth.
The 2.27% solution of disodium sulfone was obtained through the courtesy of Dr. C. A. Woodhouse from Dupont of Delaware, Md.

TABLE V.
Effect of Sodium Sulfamethazine on *S. agalactiae* and *E. coli*.

| Mg % conc. of drug | Hours incubation | *St. agalactiae* strains | | | | *E. coli* |
|---|---|---|---|---|---|---|
| | | 1723 | MP5 | 24D | N-6 | MP 8 |
| 2500 | 24 | — | — | — | — | — |
| | 48 | + | + | + | + | + |
| 250 | 24 | 3+ | + | 3+ | 2+ | + |
| | 48 | 3+ | 3+ | 3+ | 3+ | 3+ |
| 25 | 24 | 3+ | 3+ | 3+ | 2+ | + |
| | 48 | 3+ | 3+ | 3+ | 3+ | 3+ |
| 2.5 | 24 | 3+ | 3+ | 3+ | 2+ | + |
| | 48 | 3+ | 3+ | 3+ | 3+ | 3+ |
| Control | 24 | 3+ | 3+ | 3+ | 3+ | 3+ |

1723, MP5, 24D, and N-6; *S. agalactiae* cultures from cows with chronic mastitis.
MP8—*E. coli* culture from a cow with acute mastitis.
— No change in milk.
+ Slight acid in milk.
2+ Acid in milk.
3+ Acid and coagulation in milk.

were active against *E. coli, Br. abortus, Str. agalactiae,* and *Ps. aeruginosa*. They appeared to have a wider range of action than the other sulfonamides. Sulfapyridine and sulfamethazine were slightly less active. Sulfone was much less active against the gram negative organisms. The strains of *S. aureus* and *S. typhimurium* were resistant to the sulfonamides. In Table IV the effect of disodium sulfone is presented. The concentration required for inhibition of growth was more than 227 mg % for all 7 strains. Disodium sulfone was more active against *Str. agalactiae* than against the other organisms. As shown in Table V sulfamethazine sodium inhibited the 4 *Str. agalactiae* cultures and one *E. coli* culture for 24 hours in a 2500 mg % concentration but permitted growth in this concentration in 48 hours. *E. coli* was slightly more sensitive than strains of *Str. agalactiae.*

*Discussion and summary.* Data are presented on the effect of various antibacterial agents on a representative group of organisms associated with cases of chronic and acute mastitis.

Subtilin was effective in high dilution against the gram positive organisms. Streptomycin appeared to more active against the gram positive cultures than against the gram negative organisms. Strain variations among cultures of the same species of organism may

have been responsible for the poor action of streptomycin against these particular gram negative organisms.

The strains of *S. aureus* and *S. typhimurium* were resistant to the sulfonamides employed. *Str. agalactiae* and *C. pyogenes* were susceptible to sulfapyridine, sulfone, sulfadiazine and sulfamerazine. *In vivo* study is necessary to prove these drugs of value as adjuncts to penicillin in the treatment of chronic streptococcic mastitis and so-called "summer mastitis." *E. coli* was sensitive to sulfapyridine, sulfamethazine, sulfadiazine, and sulfamerazine among which sulfamerazine and sulfamethazine showed great activity. *Br. abortus* was susceptible to sulfamethazine, sulfadiazine, and sulfamerazine. *Ps. aeruginosa* was inhibited by sulfamerazine and sulfapyridine. Against the strain of *Ps. aeruginosa* employed, sulfadiazine was the most effective antibacterial agent.

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## The Effect of Heparin on Cell Division.*

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When a living cell is incited to divide, its protoplasm first undergoes a sharp increase in viscosity. There is indeed what may be called a mitotic gelation and this gelation precedes the appearance of the mitotic spindle. Once the spindle is formed, the protoplasm reverts to its original fluid state. This sequence of stages in the protoplasm of a dividing cell has been described by all authors who have used objective methods for the determination of protoplasmic viscosity, (for discussion, see Heilbrunn;[1] Heilbrunn and Wilson[2]).

Since 1928,[1] it has been maintained that the mitotic gelation is similar to the gelation produced in protoplasm by so-called stimulating agents, and that this fundamental gelation reaction of protoplasm is in many respects similar to the gelation that occurs in vertebrate blood when it clots. As a matter of fact the protoplasm of all types of living cells is rich in thrombin or thromboplastic substances, substances which affect not only protoplasm but blood as well (compare Heilbrunn *et al.*[3] and references cited there).

If this line of reasoning is correct, it might be thought that substances which inhibit blood clotting would also prevent protoplasmic clotting in general and mitotic gelation in particular. Clearly, then, a substance like heparin is of interest in this connection.

Perhaps the best type of cell in which to observe protoplasmic clotting is the egg of the sea-urchin Arbacia. When this cell is torn or broken, as the protoplasm begins to flow out, the cell seals itself in a reaction like that which occurs when blood flows from a vessel.[4] This reaction has been called the surface precipitation reaction. Years ago, one of us attempted to show that heparin might have some effect on this surface precipitation reaction. However, no effect could be observed. Because of this failure to demonstrate an effect of heparin on the surface precipitation reaction, no further studies were made. Moreover, it was hardly to be expected

* Aided by a grant from the U. S. Public Health Service.

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