(Blumenthal: Z. Klin. Med., **28,** 223, 1895). The experiments reported here were undertaken to establish the frequency of phenol-producing strains and the distribution of these as to genus.

A total of 365 strains, isolated chiefly from contaminated water, were tested for phenol production by the Marquis reaction (Rhein: Biochem. Z., **84,** 246, 1917) after incubation for varying periods of time in casein digest medium. Positive results were obtained with 127 (34.8 per cent) within 72 hours. Negative cultures remained negative up to 30 days. Forty of the positive strains retained the ability to produce phenol when tested after one year.

Methyl red, Voges-Proskauer, and citrate utilization tests on 238 of the cultures, including all of the phenol-positives, established that 123 of the phenol producers were citrate-negative strains of *Escherichia*, whereas 4 strains were *Aerobacter*. Of the 111 phenol-negatives, 27 were citrate-positive and 3 strains were citrate-negative *Escherichia*, and 81 were *Aerobacter*. Phenol production by coliforms seems to be very much a characteristic of citrate-negative *Escherichia*.

A number of phenol-positive *Escherichia* strains failed to grow in a mineral solution with 0.1 per cent tyrosine as the source of both carbon and nitrogen. They did grow when a supplementary carbon source in the form of the sodium salt of an available organic acid was added, but failed to produce phenol within 7 days. Sufficient phenol to give a qualitative test was produced in 7 days in 1 per cent peptone water with 0.1 per cent added tyrosine, but not enough to permit of an accurate quantitative determination in culture distillates.

THE PRODUCTION OF "GIANT" CELLS OF PASTEURELLA PESTIS BY TREATMENT WITH CAMPHOR[1]

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Chemical agents with polyploidizing or mutagenic properties are commonly used to induce variation in microorganisms. Camphor, effective in producing "giant" cells in yeast, as shown by Thaysen and Morris (Nature, **152,** 526, 1943), has been successfully used to obtain "giant" cells of the plague bacillus.

A virulent strain (Shasta) of *Pasteurella pestis* was grown in Doudoroff's liquid medium (Proc. Soc. Exptl. Biol. Med., **53,** 73, 1943) and incubated at 37 C for

[1] This investigation was supported in part by grants from the Office of Naval Research, U. S. Navy.

[2] The opinions and assertions contained in this report are the private ones of the writer and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

48 hours. Inocula were then prepared by removing 1.0 ml from the culture and diluting it serially until a suspension containing approximately 60 viable cells per ml was obtained. Crystalline camphor in increasing amounts from 25 to 55 mg was dissolved in 100 ml of melted agar containing 5 per cent defibrinated horse blood and immediately poured into petri dishes. After the medium had solidified, the surface was seeded with 0.1 ml of the inoculum and incubated at 37 C for 96 hours.

Colonies on the medium containing camphor grew more slowly than colonies on the control medium. Whereas seeded control plates showed colonial growth after 48 hours, a 96-hour incubation period was required before macroscopic growth was observed on the camphorated medium. A close examination of the colonies on the control and the camphorated media did not reveal any striking differences in their morphology.

The cells from several colonies on the camphorated medium were obviously longer than the cells from control colonies and could be designated as "giant" cells. The cells from colonies grown on medium containing 65 mg per cent cam-

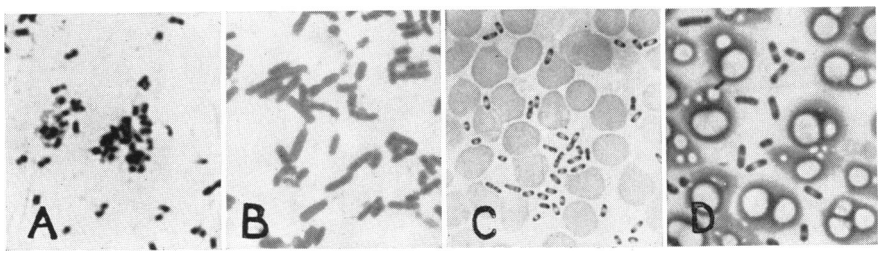

Figure 1. Normal and "giant" cells of *P. pestis*. *A:* Normal cells from control colony. *B:* "Giant" cells from colony grown on camphorated medium. *C:* Normal cells from heart's blood (mouse). *D:* "Giant" cells from lung (mouse) after 10 animal passages. Ca. × 1,300.

phor were twice as long as and slightly wider than cells from control colonies. These camphor-induced variants appeared to be stable since neither reversion nor dissociation was observed after 10 animal and 15 medium passages. The differences in cell size are graphically illustrated in figure 1. The presence of increasingly higher concentrations of camphor, moreover, seemed to reduce the striking pleomorphism characteristic of *P. pestis*.

The virulence of the "giant" and control cells was determined to ascertain whether or not this property had been altered as a result of increased cell size. Four groups of ten 8-week-old mice of the Namru strain were inoculated intraperitoneally with 0.2 ml of the appropriate dilutions of a 48-hour liquid culture of the "giant" and control cells. The "giant" cells had been subcultured on camphor-free blood agar at 48-hour intervals for a week prior to the challenge. The resulting data indicated that the "giant" cells were as virulent as the control cells. Further experiments are in progress to evaluate quantitatively the relative virulence of "giant" cells derived from several strains of *P. pestis*.

It should be noted that the effective concentration of camphor required to

induce the formation of large numbers of "giant" cells of *P. pestis* cannot be the actual quantity added to the medium since the camphor volatilized rapidly at 37 C. Consequently, rapid penetration of the camphor into the cells may be an important factor in the production of "giant" variants.

CONTROL OF MOLD CONTAMINANTS ON SOLID MEDIA BY THE USE OF ACTIDIONE

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The isolation of bacteria from contaminated specimens is often complicated by the presence of yeasts and molds. The prevention of the growth of yeasts and molds on agar plates is often desirable and sometimes a crucial necessity in problems that involve the sampling of air, surfaces, and other contaminated sources. Effective control of these contaminants has been obtained by addition, to the culture medium, of actidione[1] (an antibiotic obtained from *Streptomyces griseus*) in a concentration of 0.1 mg per ml. Whiffen (J. Bact., **56,** 283, 1948) tested the action of actidione against 12 representative species of bacteria by the broth tube method and found that none of the bacteria tested were inhibited by concentrations of actidione up to 1.0 mg per ml.

In this investigation the growth of 27 species (42 strains) of bacteria was tested by the surface plate count method on agar containing actidione in a concentration of 0.1 mg per ml of medium. Organisms that were tested, in addition to those mentioned by Whiffen, were *Alcaligenes faecalis*, *Bacillus megatherium*, *Bacillus cereus*, *Bacillus anthracis*, *Bacillus globigii*, *Brucella abortus*, *Brucella bronchiseptica*, *Brucella melitensis*, *Brucella suis*, *Chromobacterium amethystinum*, *Pasteurella tularensis*, *Pseudomonas aeruginosa*, *Salmonella paratyphi*, *Salmonella typhimurium*, *Salmonella typhosa*, *Sarcina lutea*, *Serratia indica*, *Serratia marcescens*, *Shigella dysenteriae*, *Micrococcus pyogenes* var. *albus*, *Micrococcus citreus*, *Mycobacterium smegmatis*, and *Neisseria catarrhalis*. Actidione was found to be noninhibitory in the concentration used. Also, actidione did not inhibit *Escherichia coli* T3 bacteriophage.

The antifungal action of actidione was tested against 1 strain of yeast and 7 genera (12 species) of molds that were isolated from contaminated agar plates. All were found to be inhibited completely by 0.1 mg of actidione per ml of medium. These molds and a yeast were *Alternaria* (3 species), *Aspergillus*, *Fusarium* (2 species), *Hormodendrum*, *Neurospora*, *Penicillium* (3 species), *Rhizopus*, and *Saccharomyces*.

Broth cultures of pathogenic bacteria were artificially contaminated with mix-

[1] Supplied by the Upjohn Company, Kalamazoo, Michigan.