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Mineral oils, when emulsified with materials such as soaps of petroleum sulfonates, fatty acids, abietic acid or resin, are referred to as soluble oils. These oils, mixed with water, form stable emulsions which are universally employed as coolants and lubricants for drilling, cutting, and grinding of metals. In addition to the emulsifying agent, soluble oils may also contain fatty oils, disinfectants and emulsion stabilizers.

The oils supplied by the manufacturer are sterile, but when mixed with water in the machine shop they support microbial growth (Duffet *et al.*, 1943; Fabian and Pivnick, 1953; Lee and Chandler, 1941; Page and Bushnell, 1921). In fact, contaminants from soil, floor sweepings, air, river water, and feces grow readily in these emulsions (Fabian and Pivnick, 1953).

The C. B. Dolge Company (undated pamphlet) has reported that feces and other body discharges are found in soluble oil emulsions. Duffet *et al.* (1943), Page and Bushnell (1921), and Pivnick and Fabian (unpublished data) found coliform bacteria in samples obtained from factories in the United States. Recently, Pivnick (unpublished data) has found that emulsions from factories in Great Britain, Canada, and the United States contained between 10^3 and 10^5 coliform bacteria per ml.

The relationship of fecal pollution to the spread of enteric diseases suggested that an investigation of enteric pathogens in soluble oils should be undertaken. Preliminary experiments by Okawaki (1953) showed that enteric pathogens grew well in this medium. This report is concerned with the growth of some representative enteric pathogens and *Klebsiella pneumoniae*.

MATERIALS AND METHODS

Oil emulsion. The oil sample was obtained from a local depot of a nationally recognized petroleum company. This sample contained mineral oils emulsified with soaps of petroleum sulfonate and resin. The sample was mixed with tap water to give an emulsion containing 2 per cent oil, and sterilized by autoclaving for 15 minutes at 121 C.

Cultures. Cultures of *Salmonella*, *Shigella* and *Klebsiella* were generously supplied from the stock culture collections of the University of Nebraska, University of Michigan, The Ohio State University, Dr. E. Cope of the Michigan State Department of Health, and Dr. P. R. Edwards of Chamblee, Georgia. Cultures were also obtained from the American Type Culture Collection (A.T.C.C.). The name and source of the individual cultures are given in table 1.

Inoculum. Cultures were grown in nutrient broth for 18 to 24 hours at 37 C, washed with physiological saline and inoculated into the oil emulsions. Inoculum size was varied to yield from 10^4 to 10^6 cells per ml of emulsion. The gram reaction, motility, and sugar fermentations of each culture showing growth in the emulsions were checked 4 days after inoculation.

Culture methods. The inoculated emulsions were incubated at 30 C. Aliquots were removed after 0, 4 and 8 days incubation and plated on nutrient agar. Agar plates were incubated at 30 C and colonies counted after 72 hours.

EXPERIMENTAL RESULTS

Thirty different strains of enteric pathogens and two strains of *Klebsiella pneumoniae* were tested for their ability to grow in soluble oil emulsions. Representative data from these experiments are shown in table 1.

Genus Salmonella. All strains of *S. schottmuelleri*, *S. typhimurium*, *S. oranienburg*, and *S. pullorum* grew readily in oil emulsions. Conversely, all six strains of *S. paratyphi*, and three of the four strains of *S. typhosa* tested failed to grow. The remaining strain of *S. typhosa* (obtained from Dr. P. R. Edwards) grew readily when

TABLE 1. *The growth response of several strains of enteric organisms and* Klebsiella pneumoniae *in soluble oil emulsions*

ORGANISMS	VIABLE ORGANISMS PER ML			
	0 days	2 days	4 days	8 days
Salmonella shottmuelleri (1)	16,000	375,000	16,100,000	980,000
Salmonella shottmuelleri (2)	53,000	2,610,000	84,500,000	531,000,000
Salmonella shottmuelleri (3)	3,340,000	4,200,000	4,800,000	9,200,000
Salmonella typhimurium (2)	15,500	125,000	165,000	10,000
Salmonella typhimurium (3)	90,000	640,000	1,320,000	1,920,000
Salmonella typhimurium (4) No. 9148	166,000	48,000,000	472,000,000	42,000,000
Salmonella oranienburg (2)	76,000	160,000	75,500,000	29,000,000
Salmonella oranienburg (1)	111,000	1,000,000	70,500,000	25,500,000
Salmonella pullorum (2)	2,400	620,000	35,600,000	34,000,000
Salmonella paratyphi (2)	180,000	1,300	1,000	80
Salmonella paratyphi (6)	705,000	25,000	No data	520
Salmonella typhosa (2)	120,500	12,000	18,750	90,000
Salmonella typhosa (2)	5,400,000		73,200,000	42,000,000
Shigella sonnei (3)	40,000	6,800	1,800	0
Shigella sonnei (5)	193,000	6,050	1,600	100
Shigella sonnei (4) No. 9290	662,000	18,000	3,700	1,350
Shigella dysenteriae (5)	109,500	200	0	0
Shigella dysenteriae (4) No. 9665	316,000	0	0	0
Klebsiella pneumoniae (4) No. 8044	9,100	7,800	2,600	1,700
Klebsiella pneumoniae (3)	101,000	10,400,000	41,000,000	87,000,000

Sources of cultures: (1) Michigan State Department of Health. (2) P. R. Edwards, Chamblee, Georgia. (3) University of Nebraska. (4) American Type Culture Collection. (5) University of Michigan. (6) The Ohio State University.

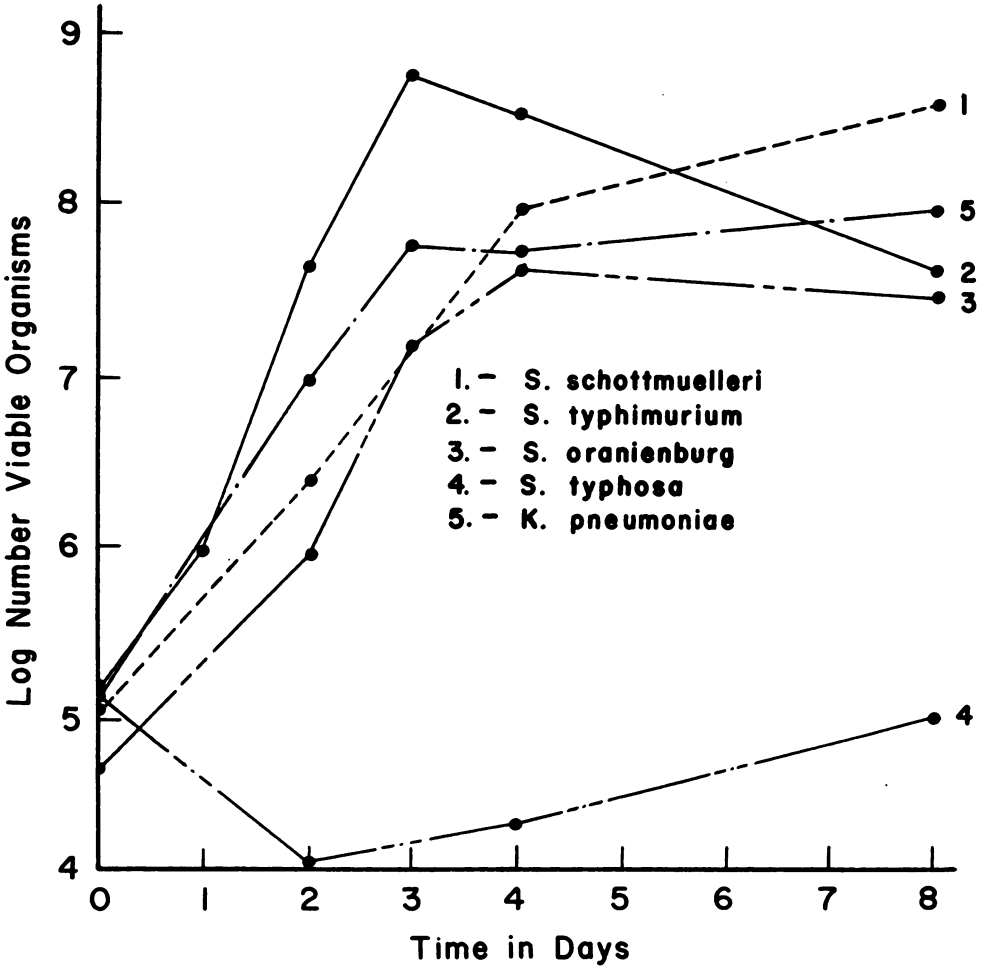

FIG. 1. A comparison of the growth responses of representative pathogens in soluble oil emulsion

a large inoculum was employed. When, however, a small inoculum (10^4 cells per ml) was used, the viable count decreased for 2 days and then slowly increased (figure 1). This strain, when typed, gave the typical serological characteristics of *S. typhosa*.

Genus Shigella. Four strains of *S. sonnei* and five strains of *S. dysenteriae* rapidly decreased in numbers and could not be detected after the tenth day of incubation.

Genus Klebsiella. The ATCC strain of *K. pneumoniae* slowly decreased in numbers, and disappeared entirely after the twenty-first day of incubation. However, the University of Nebraska strain increased steadily in numbers during the incubation period. Subcutaneous injection of small number of cells of the latter strain into white mice rapidly resulted in the death of the animals. Controls injected with heat-killed organisms survived.

Observations indicate that species within a genus and even strains of a single species differ markedly in their ability to survive and multiply in soluble oil emulsions. However, the ability of a strain to initiate growth in emulsions was not dependent upon inoculum size. When growth occurred, a peak was generally reached within 4 days, followed by a gradual decline in numbers over the remainder of the incubation period.

A comparison of the growth response, when approximately equal inocula of four different species of *Salmonella* and one species of *Klebsiella* were introduced into oil emulsions, is found in figure 1.

Discussion

The growth or survival of pathogens in petroleum emulsions presents some interesting problems from the standpoint of industrial hygiene and public health. Workers frequently expectorate into the emulsions, possibly introducing *Mycobacterium tuberculosis* and other respiratory pathogens present in the sputum. Also, judging from the frequency of coliform bacteria in emulsions used industrially, fecal material must be added inadvertently.

The emulsion contaminated by one worker may come into direct contact with the hands of many other workers. Also, hundreds of machine-shop workers may inhale spray droplets of the emulsion which arise from grinding operations. Thus, there is ample opportunity for infection of the intestinal tract, either directly, by nasal drip into the throat, or by infectious materials on the hands.

Although there has been some work done on the disinfection of soluble oil emulsions (Dow Chemical Company, 1951; Lee and Chandler, 1941; Pivnick and Fabian, 1953), it is evident from the bacterial populations of emulsions used industrially that disinfection is not a panacea. One industrial engineer, supplying a sample of used emulsion for some of our work, stated that by using a recommended disinfectant microbial growth was adequately controlled in their factory. Nevertheless, the sample contained 3,000,000 bacteria per ml with coliform bacteria present in a 10^{-5} dilution of the emulsion! Moreover, Schwartz (1949), an authority in the field of industrial dermatitis, suggests that care must be used in adding disinfectants to oil emulsions lest excessive concentrations of the disinfectant cause skin irritation. If disinfection is to solve this problem, it seems advisable that it be supervised by trained personnel.

This survey indicates that some respiratory and enteric pathogens survive and multiply in oil emulsions. As industrial employees frequently come into contact with these emulsions, it is of utmost importance to determine the fate of these and other pathogens under actual working conditions.

Acknowledgment

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Summary

Thirty strains of enteric pathogens and two strains of *Klebsiella pneumoniae* were tested for their ability to grow in a soluble oil emulsion. All strains of *Salmonella schottmuelleri*, *Salmonella typhimurium*, *Salmonella oranienburg*, and *Salmonella pullorum* grew readily. Six strains of *Salmonella paratyphi* and three strains of *Salmonella typhosa* did not grow. One strain of *S. typhosa* grew readily if a large inoculum were used. One strain of *Klebsiella pneumoniae* grew well whereas one strain died out within 21 days.

The significance of this work with respect to public health is also discussed.

REFERENCES

C. B. Dolge Company (Undated pamphlet) *Cutting Oil Sanitation.* C. B. Dolge Company, Westport, Connecticut.

Dow Chemical Co. 1951 Dowicides-industrial germicides and fungicides. Dow Chemical Co., Midland, Michigan.

Duffet N. D., Gold, S. H., and Weirich, C. L. 1943 Growth of bacteria in cutting oils. J. Bacteriol., **45,** 37.

Fabian, F. W., and Pivnick, H. 1953 Growth of bacteria in soluble oil emulsions. Appl. Microbiol, **1,** 199–203.

Lee, M., and Chandler, A. C. 1941 A study of the nature, growth and control of bacteria in cutting compounds. J. Bacteriol., **41,** 373–86.

Okawaki, M. B. 1953 A study of bacteria in oils. Thesis, Dept. of Bacteriol., University of Nebraska, Lincoln, Nebraska.

Page, C. G., and Bushnell, L. D. 1921 Oil folliculitis. J. Ind. Hyg., **3,** 62–67.

Pivnick, H. 1953 Unpublished data.

Pivnick, H., and Fabian, F. W. 1953a *Unpublished data.*

Pivnick, H., and Fabian, F. W. 1953 Methods for testing the germicidal value of chemical compounds for disinfecting soluble oil emulsions. Appl. Microbiol., **1,** 204–207.

Schwartz, L. 1949 Coolants needn't be dangerous. Am. Machinist, **93,** 90–92.