between total lipid and lytic sensitivity; i.e., the most insensitive species, *A. hartlebii*, did not contain the greatest amount of lipid (12.6%). *A. parvulus* (11.4% lipid) was not the most sensitive to lytic agents. In every case, the total amount of lipid extracted from cell walls was essentially the same as that from whole cells. When delipidized (chloroform-methanol) cell walls were subjected to a final concentration of 10 or 50 μg/ml of lysozyme, all the cell-wall suspensions of every species lysed.

Relative to lipid saturation, the higher the iodine number (Harrow et al., *Laboratory Manual of Biochemistry*, 5th ed., 1960) of the lipid was, the greater the sensitivity of the organism to lytic agents. Conversely, the more insensitive species had lower iodine numbers than lytic-sensitive species. Therefore, the degree of lipid saturation is a significant factor in the response of *Achromobacter* cells to lytic agents.

Lipid saturation was varied by growing cells at 4 C. *A. guttatus*, one of the most insensitive species to lysis, produced more unsaturated lipid (iodine number) when grown at 4 C (236) than when grown at 25 C (231), thus becoming more sensitive to lytic agents. Total lipid (15.6%) was the same when cells were grown at these two temperatures.

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EFFECT OF ARGININE ON GROWTH AND LYSIS OF *CLOSTRIDIUM BOTULINUM*

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Perkins and Tsuji (J. Bacteriol. **84**:86, 1962) found that a synthetic medium resembling the mixture employed by Williams and Blair (Bacteriol. Proc., 1950, p. 62) gave irregular growth of *Clostridium botulinum* 62A, and failed to support sporulation of the organism. A synthetic medium that contained additional amino acids supported excellent growth of the organism, and supplements of increased amounts of arginine stimulated extensive sporulation. It was suggested that the sporulation induced by increased amounts of arginine could be attributed to energy yielded by the ensuing conversion of citrulline to ornithine.

During one phase of an investigation of the nutrition of *C. botulinum* 62A (Bowers, Ph.D. Thesis, University of Texas, 1955), we had occasion to employ media resembling those used by Williams and Blair and by Roessler and Brewer (J. Bacteriol. **51**:571, 1946). Inconsistencies in growth when using these mixtures, noted by Perkins and Tsuji, were also observed by us. This has been discussed, and the medium and methods described, elsewhere (Bowers and Williams, Antonie van Leeuwenhoek J. Microbiol. Serol., *in press*). The complete medium described in that report was used in the work reported here. This medium allowed excellent growth, but failed to support sporulation of the organism. Maximal turbidity was attained in approximately 20 hr and was followed by a rapid and extensive lysis of the cells. During the early logarithmic growth phase, the cells were primarily gram-positive and ranged in diameter from moderately slender to plump. The few gram-negative cells were extremely slender. As time progressed, however, the ratio of gram-negative to gram-positive cells increased until active lysis commenced. At this time, most of the cells were very slender and gram-negative. With continuing lysis, the total number of cells decreased, as did the ratio of gram-negative to gram-positive cells. This sequence of events was similar to that described by Boroff (J. Bacteriol. **70**:363, 1955).

Turbidity of cultures in media containing in-

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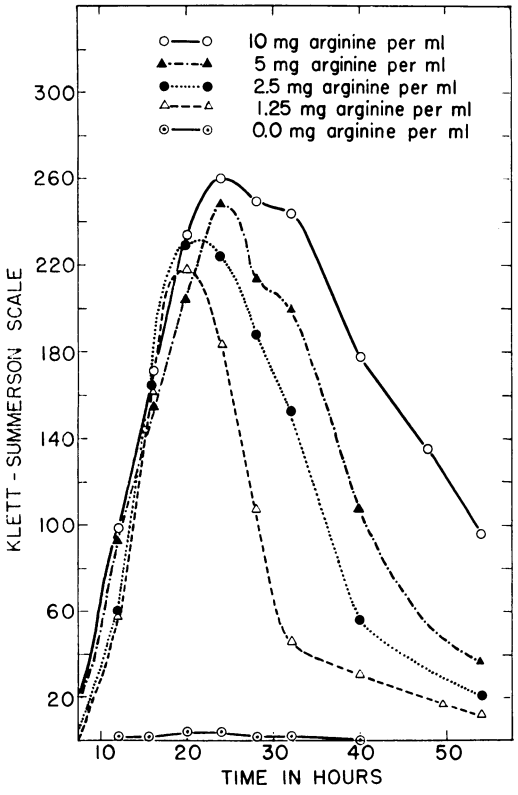

FIG. 1. *Relationship between concentration of arginine and the onset of lysis.*

creased concentrations of arginine persisted for longer periods of time (Fig. 1). The effects of arginine were maximal at concentrations of 10 to 20 mg per ml; at higher concentrations, the persistence of turbidity was evident, but maximal turbidity was decreased. The pattern of events as determined microscopically also varied with the concentration of arginine. As the concentration was increased to 10 mg per ml, an increasing tendency of the cells to retain their gram-positiveness became evident. Also, appreciable numbers of the cells contained large basophilic bodies typical of forespores when grown in the presence of higher concentrations of arginine. Refractile spores were not observed. Increased concentrations of glucose produced a similar effect; lysis was early and extensive when the medium contained 0.5% glucose, delayed and less extensive with 1.0 or 1.5% glucose. Forespores were evident when the higher concentrations of glucose were used, but fully developed spores were not found. The similarity of results obtained when increased concentrations of either glucose or arginine were added gave no indication of specificity of action with regard to sporulation. Rather, both substrates appeared to contribute to general physiological activities, including those which account for gram-positiveness and those which incidentally allow expression of inherent competence to initiate sporulation.

AEROBACTER (ENTEROBACTER) CLOACAE IN HUMAN AND ANIMAL FECES

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Among the coliform bacteria, both *Escherichia coli* and *Klebsiella* are recognized as useful indicators of fecal contamination of water or foods (Buttiaux, J. Appl. Bacteriol. **22**:153, 1959; Papavassiliou, Arch. Lebensmittelhyg. **10**:56, 1959). The incidence of *Enterobacter* (Hormaeche and Edwards, Intern. Bull. Bacteriol. Nomencl. Taxon. **10**:71, 1960) in human and animal feces has been studied recently in France (Buttiaux, Ali Zaman, and Catsaras, Ann. Inst. Pasteur **103**:101, 1962). Their results, summarized in Table 1, suggest that *Enterobacter* cannot be used as indicators of fecal contamination.

In the last few years, I studied the frequency of colicinogenic *Enterobacteriaceae* in human and animal feces (Arch. Inst. Pasteur Tunis **37**:103, 1960), and I isolated many strains of *Aerobacter cloacae* after direct plating of fecal suspensions in saline onto MacConkey agar. From each fecal specimen, no more than four colonies were examined, after selecting morphologically different colonies from MacConkey agar plates. Subcultures of the selected colonies were examined by tests detailed elsewhere (Papavassiliou, J. Appl. Bacteriol. **21**:104, 1958; Moutoussis, Samaraki-Lyberopoulou, and Papavassiliou, Acta Microbiol. Hell. **5**:26, 1960). Strains designated in this paper as *A. cloacae* were motile (stab cultures in